GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

Volume 35 MAY, 1950 Number 3

TABLE OF CONTENTS

SLIZYNSKI, B. M., Partial breakage of salivary gland chromosomes 279

DOBZHANSKY, TH., Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura* . . . 288

GRIFFING, BRUCE, Analysis of quantitative gene action by constant parent regression and related techniques 303

KHAMBANONDA, IAN, Quantitative inheritance of fruit size in red pepper (*Capsicum frutescens* L.) 322

PREER, JOHN R., Microscopically visible bodies in the cytoplasm of the "Killer" strains of *Paramecium aurelia* 344

FOGLE, H. W., and T. M. CURRENCE, The inheritance of fruit weight and earliness in a tomato cross 363

RAUT, CAROLINE, The effect of concentration of pantothenate on selection of a mutant for pantothenate synthesis in *Saccharomyces cerevisiae* 381

PUBLISHED BIMONTHLY AT MENASHA, WISCONSIN,
BY THE BROOKLYN BOTANIC GARDEN, BROOKLYN 25, NEW YORK, U.S.A.
(Date of issue, May 31, 1950)

EDITORIAL BOARD

CURT STERN, *Managing Editor*, University of California

GEORGE W. BEADLE
California Institute of Technology

WILLIAM E. CASTLE
University of California

EDWIN G. CONKLIN
Princeton University

BRADLEY M. DAVIS
University of Michigan

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
University of Illinois

GEORGE H. SHULL
Princeton University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

L. J. STADLER
U. S. Dept. Agriculture, University of Missouri

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Chicago

Volume 35 MAY, 1950 Number 3

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.25 each, postpaid.

As available, volumes 1 to 6 may be had at $12.00 each, and the later volumes at $10.00 each, postpaid. The Business Manager will supply information on request as to volumes and numbers available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

Business Correspondence, including change of address, etc., should be addressed to 450 Ahnaip St., Menasha, Wisconsin, or BROOKLYN BOTANIC GARDEN, Brooklyn 25, N.Y., U.S.A.

Remittances should be made payable to GENETICS, INC.

Entered as second-class matter, August 31, 1922, at the postoffice at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L. & R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Genetics

Golden Jubilee Supplement

To commemorate the fifty years' anniversary of Mendelism, GENETICS will publish English translations

of

MENDEL'S LETTERS TO NÄGELI

and

translations of the first publications, in 1900, of

DE VRIES, H., Sur la loi de disjonction des hybrides. Comptes Rendus **130:** 845-847.

CORRENS, C. G., Mendel's Regel über das Verhalten der Rassenbastarde. Ber. deutsch bot. Ges. **18:** 158-168.

TSCHERMAK, E. VON, Über künstliche Kreuzung bei *Pisum sativum*. Ber. deutsch. bot. Ges. **18:** 232-239.

Mendel's famous letters show the wide range of his biological interest and report on important observations not described in his published papers. In addition they give vivid impressions of Mendel's personality.

The papers by de Vries, Correns, and Tschermak illustrate the different ways in which the three rediscoverers of Mendel's work reached their conclusions and presented their results.

These translations will form a supplement of about 50 pages to the September 1950 issue of GENETICS and will be mailed to subscribers without charge.

The supplement will be of value to a wide circle of biologists, and be suitable for use in classes.

For sale as follows:

Pre-publication Prices	*Regular Prices*
(orders to be received before September 1, 1950)	1 copy $1.00
2 copies at 0.85	10 copies at 0.90
10 copies at 0.80	25 copies at 0.80
25 copies at 0.70	

Orders should be sent to

BROOKLYN BOTANIC GARDEN, BROOKLYN 25, N.Y.

Please make check payable to GENETICS INC.

Genetics: The First 50 Years

MEETINGS OF THE GENETICS SOCIETY OF AMERICA
COLUMBUS, OHIO
SEPTEMBER 11 TO 14, 1950

The GENETICS SOCIETY OF AMERICA at its annual meeting on December 29, 1949, voted to celebrate in 1950 the 50th birthday of the rediscovery of Mendel's fundamental work. Furthermore, genetics as a science began with the rediscovery of Mendel's original findings, so this proposed celebration may justifiably be called the Golden Jubilee Program of Genetics.

The meetings in 1950 of the Genetics Society are to be held at Columbus, Ohio from September 11 to 14, under the auspices of the American Institute of Biological Sciences. The first day of the meetings of the Society will be given over to the presentation of short papers describing the results of current research.

A general meeting of all the biological societies which will convene at Columbus is scheduled for the evening of Monday, September 11, at which the keynote address on "Fifty Years of Genetics" will be given by R. B. Goldschmidt, Emeritus Professor of Zoology at the University of California. This address will mark the beginning of the Golden Jubilee Program.

The papers to be given on Tuesday, September 12, will include various considerations of "Historical Aspects and the Development of Genetics." On Wednesday morning, September 13, the general topic is "The Physical Basis of the Gene," and that of the afternoon is "The Physiology of the Gene." Three papers on "Cytogenetics" are scheduled for Thursday morning, September 14, to be followed by several dealing with "Genetics and the Food Supply" and "Genetics, Medicine and Man."

The addresses of the President of the American Society of Naturalists and of the President of the American Society of Human Genetics are to be a part of the Golden Jubilee Program.

The final address will be delivered on the evening of Thursday, September 14 by Julian Huxley, until recently Secretary-General of UNESCO, under the title of "Genetics, Evolution and General Thought."

The speakers at the Golden Jubilee Program will survey the development and present status of various phases of genetics. In addition, each speaker is invited to consider probable paths of future thought and discovery. The papers are to be published as a separate volume.

PARTIAL BREAKAGE OF SALIVARY GLAND CHROMOSOMES

B. M. SLIZYNSKI

Institute of Animal Genetics, Edinburgh University, Edinburgh, Scotland

Received November 8, 1949

IN 1936 MARSHAK published a report of his experiments with very high dosage X-ray radiation of Drosophila. He treated six to eight hours old embryos with 5,000 r units and found that most of the salivary gland chromosomes showed one or more translocations. No details are given in regard to the diameter of the parts of the chromosomes involved in translocations and there is no mention of inversions or deficiencies in this series of MARSHAK's experiments. Embryos of the 10–12 hour stage treated with 5,000 r units showed deficiencies in the chromatic bands, half a band being missing in one of the homologues. In the last series of MARSHAK's experiments three day old larvae treated with 20,000 r units showed no detectable effects in their salivary gland chromosomes.

The present report deals with the effects of small dosage X-ray radiation on young embryos of *Drosophila melanogaster* and contains data from six experiments. Experiments with some other mutation producing agents show similar results.

MATERIAL AND METHODS

Flies of the Florida-4 stock were used throughout, the cultures being kept at 22°C. The first experiment was carried out in the following way: about 50 virgin females one to three days old were mated to males from the same culture bottle collected at the same time. The flies were put together at 10 P.M. and were removed from the bottle at 6 A.M. the following day. All eggs, numbering about 80, which were laid during this period were irradiated at the same time *i.e.* at 10 A.M., with a dosage of 500 r units. It may be accepted that the oldest embryos were about 12 hours old and the youngest about four hours old at the time of treatment. After irradiation the eggs were transferred to fresh food and kept at 22°C for two days; afterwards they remained at room temperature. Fully grown larvae were dissected and slides were made using the acetocarmine squash method and mounting in Euparal; some preparations were also stained by means of the Feulgen reagent. The slides were given consecutive numbers and their order thus marked approximately the age of the embryo. This method of timing, although not an exact one, was found to be very convenient in experiments concerned not with the exact age of the embryo but, for instance, with the optimum dosage of r units required to produce changes. In other experiments the ordinary method of timing was used.

Microscopical observations were made with a 1.4 NA 90× oil immersion

objective, and drawings with the aid of camera lucida and 30× eyepiece, the details being drawn in with 7× eyepieces. Microphotographs were made by MR. G. R. KNIGHT to whom the author owes gratitude.

The main difficulty in recording chromosome breaks is caused by the fact that each structural change occurs only once. This, in conjunction with the fact that even on the best slides only a few nuclei are completely analyzable is a serious disadvantage of the method when employed for the study of the frequency and distribution of chromosome breaks.

RESULTS

Structural Changes in the Salivary Gland Chromosomes

Generally speaking the results of the treatment include all known types of structural change—but the changes met with in these experiments differ fundamentally from other cases so far described, in that they do not involve the whole width of the chromosome. For instance deficiencies were observed which did not remove a complete section of the chromosome but only a longitudinal part of it so that it is still capable of pairing with its homologue along its whole length. In such cases where a piece between the points of breakage is missing the two broken ends rejoin. Since however, the elimination shortens the affected region of the chromosome a peculiar "archer's bow" structure is obtained in which the main body of a chromosome corresponds to the elastic wooden part of a bow while the affected part is stretched and may be compared to the string. Such side connections produced by rejoining of the ends are usually very thin; their diameter can best be estimated at their point of separation from the main body. Changes of this type induced in salivary gland chromosomes are called partial structural changes since they affect only a part of a chromosome. Many partial structural changes may appear in mutual combination on a single chromosome or they may involve more than one chromosome. Sometimes very complicated figures were seen where analysis was impossible.

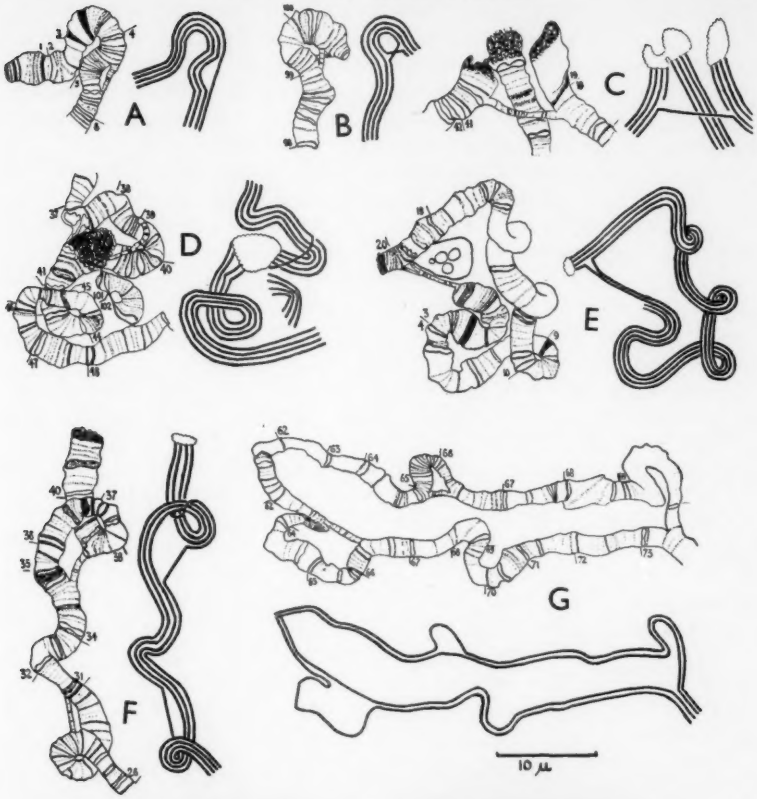
Typical partial structural changes are illustrated in figure 1, which contains camera lucida drawings and their probable interpretation; in the latter, for the sake of simplicity, the number of chromonemata has been limited to two in a univalent. In addition there are microphotographs of parts A and C.

Drawing A shows a partial deficiency of medium size in divisions 4 and 5 (BRIDGES' reference system) of the X chromosome.

Drawing B shows a partial inversion at the free end of the right arm of chromosome 3. There is no detectable partial deficiency connected with the inversion.

Drawing C shows a partial translocation between the X chromosome and the left arm of chromosome 2 in combination with the loss of both centromeres involved.

Drawing D shows a partial deficiency in the left arm of chromosome 2; the side connection unites the beginning of division 39 with a chromocentral mass. It is not possible to say to which chromosome this part of the chromocentral mass belongs.



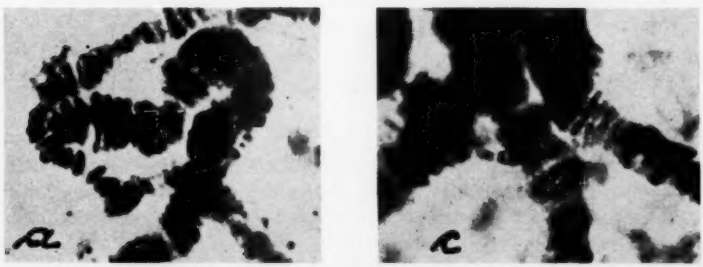


FIGURE 1.—Examples of partial structural changes. Camera lucida drawings and diagrammatic interpretations: A—deficiency, B—inversion, C—translocation, D—deficiency, E—ring formation, F—deficiency in combination in inversion, G—two deficiencies each in one of the homologues; a—microphotograph of A, c—microphotograph of C.

Drawing E shows the free end of chromosome X joined by a thin side connection to the base of the same chromosome at division 20. At the point where it joins the base, its greatly extended subdivision 1A is split into two arms; one goes over into the proximal part of the base while the other turns distally. Two ring chromosomes have thus been produced, the smaller of them being acentric.

Drawing F shows two side connections in the left arm of chromosome 2. One of them joins the body of the chromosome at divisions 38 and 35, and the other at 31 and 28. The first side connection involves a partial deficiency in combination with partial inversion, the other represents simply a partial deficiency.

Drawing G shows two largely non-paired univalents (the left arm of chromosome 3) each having a partial deficiency. One point of breakage, namely that in division 66 is common to both partial deficiencies.

It has been noticed in all the experiments that the older the embryos were at the time of treatment the thinner were the side connections. The nature of the data, however, would not justify any statistical treatment of this correlation. There are difficulties in the exact measuring of the diameters which are as a rule very much affected by the stress of extension. Those produced in one 13 hours old embryo were estimated to involve 1/16th part of the chromosome, which is however at the limit of optical visibility. The side connections observed in chromosomes belonging to cells from various parts of the same salivary gland may show a certain amount of variation in width which is probably due to the different age of the chromosomes at the time of treatment. In one of the experiments in which embryos were treated with 400 r units when 10–13 hours of age there were among 143 well determined structural changes 79 (55 percent) deficiencies, 24 (17 percent) inversions and 40 (28 percent) translocations.

Distribution of Chromosome Breaks

The X-raying of salivary gland chromosomes in Drosophila can with some limitations be used for studying chromosome breaks. No special experiments were carried out on the frequency distribution and dosage relation of partial structural changes, but during the experiments some data were collected on the subject.

The most extensive study on the distribution of chromosome breaks in all chromosomes is that of BAUER, DEMEREC and KAUFMANN (1938) who examined salivary gland chromosomes of 1,765 larvae whose male parents were treated with X-rays. They obtained 1,038 breaks. In 1939 BAUER published another paper on chromosome breaks obtaining similar results at least so far as the problems discussed here are concerned.

In one of the experiments reported here one larva which was treated with 600 r units as a 10–13 hours old embryo yielded 192 chromosome breaks. Their distribution is represented in figure 2, where it is compared with data obtained by BAUER, DEMEREC and KAUFMANN. Although the frequencies

are much higher for their material the two curves have much in common.

In figure 3, the distance between two related breaks is compared with that obtained by the same authors and also shows good agreement between the two sets of data. The low frequency at the left end of both curves, corresponding to the distances smaller than one division is probably due in both cases to the difficulties of detection and determination of such small structural changes.

The results of the present paper show that a few threads belonging to the same bundle can be broken without the destruction of the remainder. Thus it can be concluded that the action of X-rays is limited to a very small field and that the spreading of the effect of radiation does not necessarily transect the whole of the chromosome.

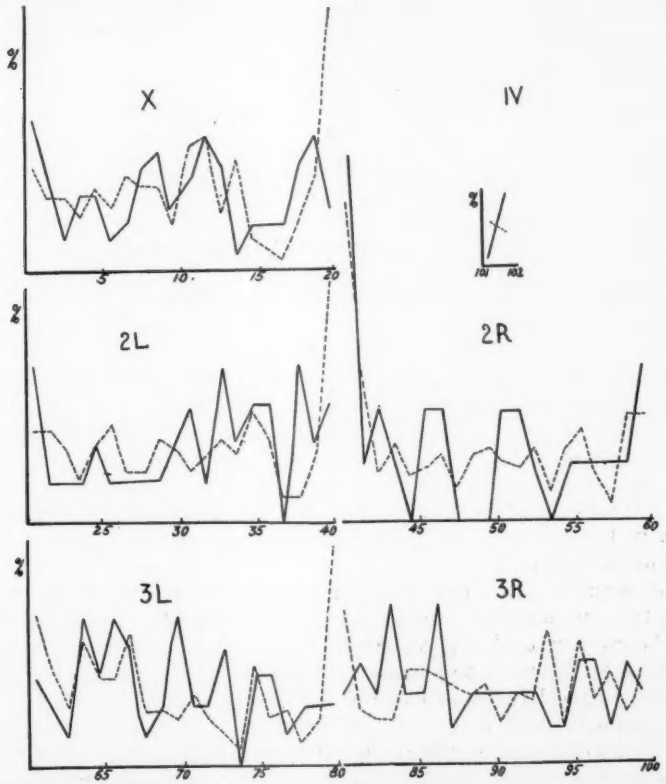


FIGURE 2.—Frequency distribution of chromosome breaks. *Solid line:* data from one larva treated with 600 r units as a 10–13 hours old embryo. *Broken line:* data from BAUER, DEMEREC and KAUFMANN (1938) from 1,765 larvae, whose male parents were treated with X-rays. *Ordinate:* frequency in percentage for each chromosome limb. *Abscissa:* BRIDGES' divisions of cytological map.

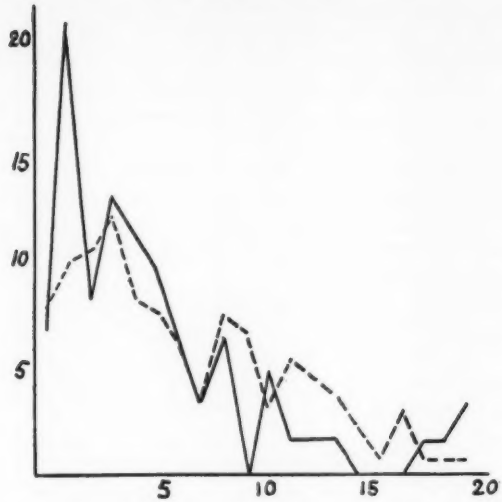


FIGURE 3.—Comparison of distances between two related breaks in the chromosomes. Data from BAUER, DEMEREC and KAUFMANN are represented by broken line, those from the present experiment by continuous line. *Abscissa:* distance in BRIDGES' divisions of salivary gland chromosomes. *Ordinate:* frequency.

DISCUSSION

There are at present three main theories of the structure of salivary gland chromosomes of Dipteran larvae.

According to the first theory in each giant chromosome there is one single thread. During the larval development there is a considerable increase in its size accompanied by coiling. Parts of these coils become dissolved away thus giving the appearance of interband spaces separating the individual bands *i.e.* those parts of the coil which did not undergo dissolution (ALVERDES 1912; KOSSWIG and SENGÜN 1947).

The second theory of their structure assumes that in a univalent chromosome there are only two chromonomata which are either directly visible or may be made visible by appropriate technique. The larger diameter of giant chromosomes is due to an accessory material which may appear in form of numerous secondary chromonemata (CALVIN, KODANI and GOLDSCHMIDT 1940, and KODANI 1942.)

The third theory is the polytene theory which postulates that the giant chromosomes are composed of a large number of threads. These are homologous and mutually equivalent *i.e.* all of the same rank. They are either at the limit of optical visibility or entirely submicroscopic. A giant chromosome is produced by repeated internal divisions of the original chromosome but there is no certainty about how and when these divisions take place (KOLTZOFF 1934; BRIDGES 1935; METZ 1935; BAUER 1935).

The results of the present paper are distinctly in favor of the polytene theory and in this way they support recent studies by GLANCY (1946) who using a refined micrurgical technique was able to demonstrate directly the polytene nature of giant chromosomes by successful isolation of a single fibril. One objection which might have been raised against her evidence is that isolation of fibrils was made in a chromosome which might have been already subject to some changes. The evidence from the present paper disposes of this objection since it shows that salivary gland chromosome can be partitioned *in vivo*.

The data collected in the present paper may partly help in determining the time at which the reproduction of the chromonemata sets in. From the evidence concerned with the thickness of side connections and its correlation with the age of the embryo at the time of treatment it may be concluded that the internal reproduction starts shortly after the end of mitotic divisions.

On the other hand it may be suggested that the relative thickness of side connections does not necessarily prove reproduction of chromonemata. The reasoning is as follows: the same number of strands is present in younger and in older chromosomes but the chromonemata of the old chromosomes are further separated laterally and besides that grow in thickness. In younger chromosomes when the chromonemata are close together several of them might be broken by one hit while in older ones where they are more apart only single chromonemata may be broken, and this would also give the results observed.

The objection is based on the assumption that in mitotic chromosomes of last anaphase the number of threads in each chromosome is the same as in the mature salivary gland chromosomes.

However, BAUER (1935) has shown in Chironomus that salivary gland chromosomes consist of threads in a considerable number which is much higher than that ever assumed for mitotic chromosomes.

And since the side connections described in the present paper may be less than 1/16th of the diameter of a chromosome the number of threads present at the last mitotic division would have to be at least 16 if not much more. Similar evidence is supplied by GLANCY's data (1946).

Two estimations made by MULLER (1935) give the average diameter of a single chromonema as 0.012 microns (one estimate—0.02, the other—0.004). The diameter of a univalent salivary gland chromosome is about 3 microns *i.e.* about 250 times this figure. Therefore a single chromonema would have to increase hundreds of times in thickness to attain the size of a fully matured salivary gland chromosome. If there were two, four or even eight chromonemata in the original chromosome the rate of their postulated increase would still have to be very high.

On the other hand it could be that the chromonemata do not grow but that the insertion of accessory material between the chromonemata is responsible for the final diameter of salivary gland chromosomes. In such a case one would expect to detect these accessory materials even optically since their amount would have to be very large.

The large diameter of salivary gland chromosomes lends itself more readily

to explanation on a theory of internal multiplication than by such enormous growth rates in individual chromonemata or the insertion of such large amounts of accessory material. JACOBJ'S data, quoted by MULLER (1941), strongly suggest that there is a periodic increase in the number of elements during the growth of a nucleus in salivary glands.

If the number of threads both in mitotic and in salivary gland chromosomes is constant it may be either small or large. A large number does not agree with recent ideas of the structure of mitotic chromosomes while the small number does not agree with polytene structure of salivary gland chromosomes. It may be therefore safely inferred that the number of threads is increasing during the development of salivary glands.

The question of internal multiplication of salivary gland chromosomes can not be directly and finally solved on the evidence presented here. Perhaps a new experiment with two successive treatments separated by a short time interval could produce a partial structural change in the side connections themselves thus proving beyond doubt that these consist of minor threads which increase in number during the development.

SUMMARY

Low dosage X-ray treatment (400–600 r units) of *Drosophila melanogaster* embryos when applied in early developmental stages produces in the salivary gland chromosomes partial structural changes affecting only a few of the chromonemata of which the chromosome is composed.

Partial structural changes include deficiencies, inversions, translocations and ring chromosomes; partial structural changes may also appear in mutual combination.

Structural changes in which a few fibrils are thus separated *in vivo* from the remaining mass of the chromonemata form confirmatory experimental evievidence of the polytene nature of salivary gland chromosomes of Diptera.

LITERATURE CITED

ALVERDES, F., 1912 Die Kerne in den Speicheldrüsen von Chironomus Larvae. Arch. Zellforsch. **9**: 168–204.

BAUER, H., 1935 Der Aufbau der Chromosomen aus den Speicheldrüsen von Chironomus Thummi Kiefer. Z. Zellforsch. **23**: 280–313.

1939 Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. Chromosoma **1**: 343–389.

BAUER, H., M. DEMEREC, and B. P. KAUFMANN, 1938 X-ray induced chromosomal alterations in *Drosophila melanogaster*. Genetics **23**: 610–630.

BRIDGES, C. B., 1935 Salivary chromosome maps. J. Hered. **26**: 60–64.

CALVIN, M., M. KODANI, and R. GOLDSCHMIDT, 1940 Effects of certain chemical treatment on the morphology of salivary gland chromosomes and their interpretation. Proc. nat. Acad. Sci., Wash. **26**: 340–349.

GLANCY, E. A., 1946 Micrurgical studies on Chironomus salivary gland chromosomes. Biol. Bull. **90**: 71–87.

JACOBI, W., 1929 Das geometrische Prinzip der "Moebiusringe" im Chromosomenmechanismus der heterotypischen Mitose und seine Bedeutung für Vererbung und Geschwulstentstehung. Roux' Archiv **120**: 56–191 (quoted by Muller, 1941, Cold Spring Harbor Symp. Quant. Biol. **9**: 54.)

KODANI, M., 1942 The structure of salivary gland chromosomes. J. Hered. **33**: 114–133.

KOLTZOFF, 1934 quoted by Metz, 1941 Cold Spring Harbor Symp. Quant. Biol. **9**: 24.

KOSSWIG, C., and A. SENGÜN, 1947 Neuere Untersuchungen über den Bau der Riesenchromosomen der Dipteren. Rev. Fac. Sci. Istanbul **12**: 107–121.

MARSHAK, A., 1936 The structure of the chromosomes of the salivary gland of *Drosophila melanogaster*. Amer. Nat. **70**: 181–184.

METZ, C. W., 1935 Structure of the salivary gland chromosomes in Sciara. J. Hered. **26**: 177–188.

MULLER, H. J., 1935 On the dimensions of chromosomes and genes in Dipteran salivary glands. Amer. Nat. **69**: 405–411.

GENETICS OF NATURAL POPULATIONS. XIX. ORIGIN OF HETEROSIS THROUGH NATURAL SELECTION IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

THEODOSIUS DOBZHANSKY
Columbia University, New York City

Received November 8, 1949

IN MOST parts of the geographic distribution of *Drosophila pseudoobscura*, chromosomal types which differ in gene arrangement in the third, the X, and, less frequently, in other chromosomes occur in the populations. The diversity of the gene orders is due to inversion of chromosome sections. Since the chromosomal types interbreed at random, inversion heterozygotes and homozygotes are found in nature. The observation that seasonal changes in relative frequencies of third-chromosome gene arrangements take place in populations of Piñon Flats, on Mount San Jacinto, in California, has suggested that these gene arrangements affect the adaptive properties of their carriers (DOBZHANSKY 1943). Indeed, experimental populations kept in so-called "population cages" have shown that the inversion heterozygotes which carry two third chromosomes with different gene arrangements derived from the same population are superior in fitness to the corresponding homozygotes (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1947 a, b). The chromosomal polymorphism is, accordingly, adaptive and it is balanced. Natural selection preserves all chromosomal types in a population at equilibrium frequency levels determined by their relative adaptive values. Intrapopulational heterosis is thus maintained.

Inversion heterozygotes which carry two third chromosomes with different gene arrangements derived from the population of Keen Camp on Mount San Jacinto (some 13 miles from Piñon Flats) or from the population of Mather (300 miles away from Piñon Flats) also possess, with one exception, higher fitness than do the homozygotes. Populations of these three localities consist mostly of the same chromosomal types. Experiments with population cages showed, however, that the adaptive values of the heterozygotes and homozygotes for the same inversions are not the same in the Piñon, Keen, and Mather populations (DOBZHANSKY 1948a, b). The structural differences between the chromosomes evidently do not account for the heterosis observed in the inversion heterozygotes. It is more likely that the heterosis is an outcome of interaction of polygene complexes carried in the chromosomes with different gene arrangements. These polygene complexes are fitted together, or "coadapted," by natural selection in the course of the evolutionary process. Heterosis is a result of natural selection (DOBZHANSKY 1949).

The process of mutual adjustment of gene contents may, however, have taken place only between chromosomes which occur in the same population. Chromosomes of different populations need not be coadapted, unless these

populations often hybridize. Natural selection may have produced different polygene complexes in different populations, coadapted within but not between the populations. If this hypothesis is correct, inversion heterozygotes which carry two chromosomes derived from different populations may not show as much heterosis as is observed within these populations themselves. The present article describes experiments devised to test this hypothesis. The hypothesis has withstood the tests.

MATERIAL AND TECHNIQUE

The strains used for the purposes of the present study were mostly the same as those used previously (DOBZHANSKY 1947a, 1948b). They were derived from wild flies collected in the Piñon Flats and Mather localities in California. In addition, 12 strains collected by PROFESSOR H. SPEITH at Santa Barbara, Chihuahua, Mexico, were employed. Since wild flies are usually heterozygous for gene arrangements, pair matings were made and their progenies examined cytologically to select strains homozygous for the Standard (ST), Arrowhead (AR), and Chiracahua (CH) gene arrangements in the third chromosome (see DOBZHANSKY 1948 for further comment on this procedure). In all, 15 ST, 12 AR, and 16 CH strains of Piñon Flats origin, 8 ST, 10 AR, and 16 CH of Mather origin, and 12 CH strains of Mexican origin were employed.

Population cages of the type described by WRIGHT and DOBZHANSKY (1946), but somewhat improved in construction, were used. All experimental cages were kept at 25° C, in incubators or in a constant temperature room, with no light except that given off by the heating bulbs in the incubators.

VIABILITY OF HYBRIDS BETWEEN STRAINS OF DIFFERENT GEOGRAPHIC ORIGIN

Flies from 15 strains of Piñon Flats origin, homozygous for CH, were crossed, in regular culture bottles, to flies from 10 strains of Mather origin, homozygous for AR chromosomes. In some bottles CH flies were used as females and AR as males, and in others the reciprocal cross was made. In either case, the progeny are AR/CH heterozygotes.

On February 3, 1947, 1,217 of such heterozygotes, about equal numbers from different crosses, were placed in population cage No. 45. Their offspring (the first generation developing in the cage) will start the development consisting of 25 percent of AR/AR homozygotes, 50 percent AR/CH heterozygotes, and 25 per-cent CH/CH homozygotes. The crowding and vigorous competition in the population cage causes, however, so great a mortality between the egg and the adult stages that only a fraction of the eggs deposited give rise to adult flies. If the viability of the homo- and heterozygotes is unequal, the mortality may be differential, and the ratios among the adult flies may be different from 1:2:1. The hypothesis of differential mortality was tested by examination of the constitution of the adult flies emerging in the cage in the first generation.

Early in March, several cups crowded with pupae and mature larvae were withdrawn from cage No. 45, and the young adult flies emerging in these cups were isolated, so that the females remained virgin. These flies were outcrossed

TABLE 1

Observed numbers and viability quotients of homozygotes and heterozygotes for chromosomes with ST, AR, and CH gene arrangements of Mather (M) and Piñon Flats (P) origin. The viability of heterozygotes is taken to be unity.

	CAGE NO. 45			CAGE NO. 46		
	AR^M/AR^M	AR^M/CH^P	CH^P/CH^P	ST^P/ST^P	ST^P/AR^M	AR^M/AR^M
Observed ♀♀	34	46	10	13	41	33
Viability	1.40	1	0.39	0.58	1	1.56
χ^2	3.70	—	8.57	3.57	—	5.73
Observed ♂♂	41	65	19	21	58	45
Viability	1.22	1	0.54	0.67	1	1.47
χ^2	1.19	—	7.03	3.16	—	6.47
Observed Total	75	111	29	34	99	78
Viability	1.28	1	0.47	0.63	1	1.51
χ^2	4.29	—	15.32	6.63	—	12.15

	CAGE NO. 47			CAGE NO. 48		
	ST^M/ST^M	ST^M/AR^P	AR^P/AR^P	ST^M/ST^M	ST^M/CH^P	CH^P/CH^P
Observed ♀♀	31	49	20	37	53	10
Viability	1.19	1	0.76	1.32	1	0.33
χ^2	0.93	—	1.42	2.74	—	11.75
Observed ♂♂	38	51	11	29	53	18
Viability	1.41	1	0.39	1.03	1	0.63
χ^2	4.34	—	9.60	0.01	—	3.14
Observed Total	69	100	31	66	106	28
Viability	1.31	1	0.57	1.18	1	0.48
χ^2	4.67	—	9.28	1.63	—	14.32

	CAGE NO. 49					
	ST^P/ST^P	ST^P/CH^M	CH^M/CH^M			
Observed ♀♀	14	19	2	—	—	—
Viability	1.40	1	0.17	—	—	—
χ^2	1.49	—	6.51	—	—	—
Observed ♂♂	59	81	22	—	—	—
Viability	1.38	1	0.49	—	—	—
χ^2	5.85	—	10.34	—	—	—
Observed Total	73	100	24	—	—	—
Viability	1.38	1	0.43	—	—	—
χ^2	7.35	—	16.05	—	—	—

in individual cultures to known AR/AR homozygotes; chromosomes were examined in salivary glands of six larvae from each culture. Now, if the parent of the culture was an AR/AR homozygote, all larvae will also be AR/AR. If the tested individual was CH/CH, all larvae will be CH/AR. Finally, if the tested parent was AR/CH, half of the progeny should be AR/AR and half AR/CH; with six larvae examined, the probability is 31:32 that the two chromosomal types will actually be seen, but one thirty-secondth of the heterozygotes will be misclassified as homozygotes. The results of the tests of 90 males and 125 females from cage No. 45 are shown in table 1.

If no differential mortality occurred in the cage, the AR/AR and CH/CH classes should have been equally frequent, and half as frequent as the AR/CH class. In reality, the heterozygotes AR/CH are less than twice as frequent as AR/AR, but more than twice as frequent as CH/CH (table 1). In the environment of the population cage, the viabilities of these three classes are AR/AR >AR/CH>CH/CH. The viability of AR/CH may be taken to be unity, and the viabilities of the other classes expressed in relation to that of the heterozygotes. Let the numbers of AR/AR, AR/CH, and CH/CH be denoted a, b, and c respectively. The expected numbers of AR/AR and CH/CH are, then b/2, and the viabilities (viability quotients) of these classes 2a/b and 2c/b respectively. Because of the misclassification of 1/32 of the homozygotes (see above), the computations are slightly more complex, namely:

$$\text{Viability of AR/AR} = \frac{(a - b/62)}{(32b/62)} = \frac{62a - b}{32b},$$

and

$$\text{Viability of CH/CH} = \frac{(c - b/62)}{(32b/62)} = \frac{62c - b}{32b}.$$

It is important to test the statistical significance of the observed deviations of the viability quotients from unity, i.e., from the viability of the heterozygotes. This can be done by computing chi-squares as follows:

$$\text{For AR/AR, } \chi^2 = \frac{(a - 33b/62)^2}{(33b/62)} = \frac{(62a - 33b)^2}{2{,}046b},$$

and

$$\text{For CH/CH, } \chi^2 = \frac{(c - 33b/62)^2}{(33b/62)} = \frac{(62c - 33b)^2}{2{,}046b}.$$

Each of these chi-squares has one degree of freedom; the two chi-squares are independent of each other, because either homozygote may be more or less viable in relation to the heterozygotes. The viability quotients and their chi-squares are entered in table 1. The CH^P/CH^P homozygotes are quite significantly inferior to the AR^M/CH^P heterozygotes but the AR^M/AR^M homozygotes are superior to the heterozygotes in viability.

Four further experiments were made in which the viability of heterozygotes which carried third chromosomes of Mather and of Piñon Flats origin with different gene arrangements was compared with the viab lities of the corresponding homozygotes. In all cases, flies from several strains homozygous for desired chromosomes were intercrossed in culture bottles; between one and two thousand F_1 hybrid flies were introduced into each population cage. Among the zygotes formed in the population cages in the next generation, one-half were necessarily heterozygous for the gene arrangements, and one-quarter belonged to either of two homozygous classes. The ideal 1:2:1 ratio was, however, disturbed among the adult flies developing in the cages because of the unequal viabilitles of the three types of offspring, and, consequently, because of unequal elimination. The empirical ratios were determined by testing, for chromosomal constitution, samples of adult flies which hatched from pupae formed in the population cages. This entailed the laborious but unavoidable crossing of the flies to be tested in individual cultures to chromosomally known flies, and examining the salivary gland chromosomes in six larvae in each progeny.

Population cage No. 46 contained ST chromosomes of Piñon Flats origin and AR chromosomes of Mather origin. The reciprocal combination, ST from Mather and AR from Piñon Flats, was made in cage No. 47. Cage No. 48 had ST chromosomes from Mather and CH from Piñon Flats, and cage No. 49 ST chromosomes from Piñon Flats and CH from Mather. The results are summarized in table 1, in which the observed numbers of females and males of different chromosomal constitutions are given. Taking the viability of the heterozygotes to be 1 in all cases, the viability quotients of the homozygotes are determined with the aid of the formulae given above. Chi-squares, testing the significance of the deviations between the observed viability quotients and unity, are also given.

In every experiment, one of the homozygotes is sharply inferior in viability to the heterozygote (quotients 0.17 to 0.76). In cages Nos. 45, 48, and 49, the poorly viable homozygote is CH/CH, in No. 48, it is ST/ST, and in No. 47, it is AR/AR. The inferiority is statistically quite significant for every total (chi-squares 6.63 to 16.05), but not always for both sexes. In four experiments out of five, the viability is lowered in females relatively more than it is in males. Every experiment shows also that one of the homozygotes is more viable than the heterozygote (quotients 1.03–1.56), although the superiorities are not always significant statistically and one gains the impression that the heterozygotes are closer in viability to the more viable than to the less viable homozygotes.

As stated in the introduction, Mather and Piñon Flats are about 300 miles distant from each other, and both are located in California. It seemed desirable to study the behavior in heterozygotes of chromosomes coming from geographically more remote places. Strains homozygous for CH gene arrangement from Mexico were, accordingly, crossed to ST and AR strains from Piñon Flats. F_1 hybrid flies were placed in cages Nos. 55 and 56, and the chromosomal constitution of the adult flies of the first generation developing in the cages

was examined as described for cages Nos. 45–49. The results are summarized in table 2.

Taken at face value, cage No. 55 behaved like the cages containing chromosomes from different localities in California, *i.e.*, the viability of the heterozygotes proved to be intermediate between the homozygotes (ST^P/ST^P $>ST^P/CH^M>CH^M/CH^M$). The differences are, however, mostly below the conventional level of statistical significance. But in cage No. 56, a very interesting result is obtained, *i.e.*, one of the homozygotes (AR^P/AR^P) is quite significantly more viable, while the other homozygote (CH^M/CH^M) has an equal or a slightly greater viability than the heterozygote.

TABLE 2

Observed numbers and viability quotients of homozygotes and heterozygotes for chromosomes with AR, ST, and CH gene arrangements of Piñon Flats (P) and Mexican (M) origin. The viability of heterozygotes is taken to be unity.

	CAGE NO. 55			CAGE NO. 56		
	ST^P/ST^P	ST^P/CH^M	CH^M/CH^M	AR^P/AR^P	AR^P/CH^M	CH^M/CH^M
Observed ♀♀	47	48	25	29	29	17
Viability	1.87	1	0.98	1.84	1	1.10
χ^2	18.01	—	0.01	11.92	—	0.16
Observed ♂♂	34	74	32	55	75	47
Viability	0.86	1	0.81	1.39	1	1.18
χ^2	0.74	—	1.39	5.70	—	1.26
Observed Total	81	122	57	84	104	64
Viability	1.26	1	0.87	1.53	1	1.16
χ^2	3.97	—	0.97	14.83	—	1.35

STRUCTURAL AND GENE HYBRIDITY

In the experiments so far described, inversion heterozygotes with two third chromosomes of different geographic origin were compared to inversion homozygotes with two third chromosomes of the same origin. In all these experiments, the inversion heterozygotes failed to show the superior viability ("heterosis") which heterozygotes usually have within a geographic strain. Now, it is possible that the gene contents of the chromosomes of different geographic origin are different regardless of the gene arrangement. For example, ST and AR chromosomes of the Mather population may have certain genes in common which distinguish them from the chromosomes of the Piñon Flats population. If so, in the experiments so far described, we compared inversion heterozygotes, which were also heterozygous for geographical gene complexes, with inversion homozygotes which carried third chromosomes of similar geographic origin. Following a suggestion made in a conversation with PROFESSOR H. J. MULLER, two other experiments were arranged, in which the inversion

homozygotes carried third chromosomes of different geographic origin, while inversion heterozygotes carried chromosomes of similar geographic origin.

Strains with ST chromosomes of Piñon Flats origin were outcrossed, in culture bottles, to AR strains of Mather origin. The F_1 flies from this cross are, of course, ST^P/AR^M heterozygotes. In other bottles, AR strains of Piñon Flats origin were crossed to ST strains of Mather origin. In these bottles, the F_1 flies are ST^M/AR^P heterozygotes. Females ST^P/AR^M were then crossed to ST^M/AR^P males and the mixture placed in population cage No. 50. Population cage No. 51 received ST^M/AR^P females and ST^P/AR^M males. The first generation developing in either population cage will then contain, at the start, four genotypes in equal numbers, namely, 1 ST^P/ST^M:1 ST^P/AR^P:1 ST^M/AR^M:1 AR^M/AR^P. The inversion homozygotes carry genes of Piñon Flats and of Mather origin, while the heterozygotes have, in their third chromosomes, either Piñon Flats or Mather genes (since the inversions strongly suppress crossing over, the recombinations in these chromosomes may be disregarded, cf. DOBZHANSKY and EPLING 1948). The chromosomal constitution of the adult flies hatching in cages Nos. 50 and 51 was determined as usual. Table 3 summarizes the data.

TABLE 3

Observed numbers and viability quotients of homozygotes and heterozygotes for chromosomes with AR and ST gene arrangements of Piñon Flats (P) and Mather (M) origin. The viability of the heterozygotes is taken to be unity.

		ST^M/ST^P	ST^P/AR^P AND ST^M/AR^M	AR^M/AR^P
Cage No. 50	Observed ♀♀	32	64	17
	Viability	0.94	1	0.48
	χ^2	0.13	—	8.55
Cage No. 50	Observed ♂♂	30	57	18
	Viability	0.99	1	0.64
	χ^2	0.01	—	5.03
Cage No. 51	Observed ♀♀	29	58	8
	Viability	0.94	1	0.24
	χ^2	0.11	—	16.94
Cage No. 51	Observed ♂♂	23	67	20
	Viability	0.63	1	0.54
	χ^2	4.50	—	21.46
Total	Observed	114	246	63
	Viability	0.87	1	0.46
	χ^2	2.19	—	35.25

The results in cages Nos. 50 and 51 are clearly different from all previous experiments in which chromosomes of different geographic origin were involved. They are more nearly similar to those where the chromosomes came

from the same locality. The heterozygotes as a class are superior in viability to one of the homozygous classes (AR/AR), and, taking the data at face value, also superior to the other homozygotes (ST/ST), although the viability quotients do not significantly differ from unity in the latter case. Heterozygosis for ST and AR third chromosomes from the same population results in an increase in viability, even in the presence of a geographically mixed genetic background of chromosomes other than the third.

NATURAL SELECTION IN HYBRID POPULATIONS

Analysis of the first generation of flies developed in population cages has shown that heterozygotes which carry two third chromosomes of different geographic origin are inferior in viability to at least one of the homozygotes. Now, in a population in which the heterozygotes for a gene or a gene arrangement are superior to both homozygotes, natural selection leads to an equilibrium, at which all the gene alleles or variant gene arrangements continue to be present with certain predictable frequencies. This was observed in earlier experiments on competition of third chromosomes of *Drosophila pseudoobscura* in population cages (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY 1948). If, however, the heterozygotes are equal or inferior in fitness to at least one of the homozygotes, no equilibrium should be reached, and the alleles or gene arrangements which form superior homozygotes should eventually crowd out and eliminate their competitors. The population cages No. 45–49 were, therefore, continued for 13–14 months. At intervals of one to two months, samples of eggs deposited in the cages were taken, larvae coming from these eggs allowed to develop at optimal conditions in culture bottles, and 150 of the larvae from each sample used for examination of the salivary gland chromosomes. A "sample" consisted of six "subsamples" of 25 larvae each, grown from eggs taken on six successive days (for details of this technique see WRIGHT and DOBZHANSKY 1946, pp. 131–132). The resulting data are presented in tables 4–6.

TABLE 4

Percentage frequencies of AR chromosomes of Mather origin (AR^M) and CH chromosomes of Piñon Flats origin (CH^P) in population cage No. 45. E=early; M=middle; L=late.

TIME	AR^M	CH^P	TIME	AR^M	CH^P
February 2, 1947	50.0	50.0	E. August, 1947	82.3	17.7
E. March, 1947	60.7	39.3	E. September, 1947	83.7	16.3
E. April, 1947	61.3	38.7	M. November, 1947	88.7	11.3
E. June, 1947	73.3	26.7	L. January, 1948	89.7	10.3
E. July, 1947	76.3	23.7	L. March, 1948	91.0	9.0

Cage No. 45 was started in February 1947 with equal numbers of AR chromosomes of Mather origin and CH chromosomes of Piñon Flats origin. Six months later, in early July, AR chromosomes had reached the frequency of 76 percent and CH declined to 24 percent. By March 1948, AR rose to 91 percent and CH declined to 9 percent respectively. These results are clearly different

TABLE 5

Percentage frequencies of ST and AR chromosomes of Mather (M) or of Piñon Flats (P) origin in population cages Nos. 46 and 47. E=early; M=middle; L=late.

TIME	CAGE NO. 46		CAGE NO. 47	
	ST^P	AR^M	ST^M	AR^P
February 2, 1947	50.0	50.0	—	—
E. March, 1947	39.6	60.4	50.0	50.0
E. April, 1947	62.0	38.0	59.5	40.5
M. June, 1947	66.0	34.0	73.7	26.3
M. July, 1947	68.3	31.7	78.3	21.7
M. August, 1947	74.3	25.7	79.3	20.7
M. November, 1947	72.0	28.0	76.8	23.3
L. January, 1948	75.3	24.7	75.0	25.0
L. March, 1948	79.0	21.0	81.0	19.0

from what is observed in competition of AR and CH chromosomes from the same population. In Piñon Flats populations, an equilibrium is established at about 79 percent of AR, and in Mather populations at 53 percent of AR (DOBZHANSKY 1948[1]). From the data in table 4 the following estimates of the adaptive values (W) and selection coefficients (s and t) of the chromosomal types in cage No. 45 may be derived (cf. WRIGHT and DOBZHANSKY 1946 for method):

Genotype	*W*	
AR_MAR^M	1.02	$s=-0.02$
AR_MCH^P	1.00	$t=0.43$
CH_PCH^P	0.57	

The adaptive value of AR^M/AR^M homozygotes is equal or slightly superior to that of the heterozygotes, and clearly superior to the CH^P/CH^P homozygotes. The fact that CH^P chromosomes have not been eliminated in cage No.

TABLE 6

Percentage frequencies of ST and CH chromosomes of Mather (M) and Piñon Flats (P) origin in population cages Nos. 48 and 49. E=early; M=middle; L=late.

TIME	CAGE NO. 48		CAGE NO. 49	
	ST^M	CH^P	ST^P	CH^M
March 13, 1947	50.0	50.0	50.0	50.0
M. April, 1947	59.5	40.5	62.4	37.6
L. June, 1947	76.3	23.7	79.3	20.7
L. July, 1947	80.7	19.3	82.3	17.7
L. August, 1947	82.0	18.0	85.7	14.3
L. November, 1947	88.0	12.0	90.7	9.3
E. February, 1948	90.3	9.7	92.0	8.0
L. March, 1948	92.0	8.0	93.0	7.0

[1] In this paper, the adaptive values, W, of the AR/AR and CH/CH types are incorrectly given as 0.81 and 0.60 respectively (p. 598); the correct figures are 0.48 for AR/AR and 0.40 for CH/CH.

45 is not surprising. With the adaptive values indicated the experiments must be continued for at least three years longer to have a reasonable chance of CH^P chromosomes becoming extinct.

Cages Nos. 48 and 49 were started on March 13th, 1947, with equal numbers of ST and CH chromosomes of Mather and Piñon Flats origin (table 5). As early as July of the same year, the frequency of ST rose to about 80 percent, and by March, 1948, to more than 90 percent. In pure Piñon Flats populations, the equilibrium of ST and CH chromosomes is established at about 74 percent ST, and in pure Mather populations at about 77 percent ST (DOBZHANSKY 1948). From the data in table 5 the following estimates can be computed:

	Genotype	*W*	
Cage No. 48	ST^M/ST^M	1.025	$s=-0.025$
	ST^M/CH^P	1.00	$t=0.36$
	CH^P/CH^P	0.64	
Cage No. 49	ST^P/ST^P	1.025	$s=-0.025$
	ST^P/CH^M	1.00	$t=0.61$
	CH^M/CH^M	0.39	

The ST/ST homozygotes are equal or slightly superior in adaptive value to the heterozygotes, while the CH/CH homozygotes are much inferior. At the end of the experiments, the CH chromosomes were rare in cages Nos. 48 and 49, but much longer observation would be needed to see them eliminated completely.

The adaptive values of the chromosomal types estimated from experiments Nos. 45, 48, and 49 may be compared with the estimates of the relative viability of the same chromosomal types. The relative viability has been computed on the basis of the deviations from the binomial square rule observed among the first generation adults in the same population cages (p. 290). The comparison shows that the adaptive values and the viability quotients are not identical. Although either set of estimates is subject to large experimental errors, it appears to be more than a coincidence that the heterozygotes are about equal in adaptive value to the fittest homozygotes, while the viability of the heterozygotes is definitely intermediate between the homozygotes. That such discrepancies are real and not caused by experimental errors is clearly attested by the outcome of experiments Nos. 46 and 47 (table 6). It may be recalled that the viability quotients of the chromosomal types in cage No. 46 were found to be $AR^M/AR^M > AR^M/ST^P > ST^P/ST^P$. On this basis, one might expect that the frequency of ST chromosomes in this cage would decline with time, and eventually AR chromosomes would be the only ones present. The experiment has given a quite different result. The frequency of ST gradually rose, until an equilibrium was approached in the vicinity of 80 percent ST[2]). In cage No. 47, a trend toward an equilibrium at

[2] The value 39.6 percent ST in cage No. 46 recorded for March, 1947, in table 6 is based on the analysis of the chromosomal constitution of the adult flies of the first generation hatching in this cage (see data in table 1). It is, accordingly, not quite comparable with the other values in table 6, which are based on examination of larvae hatching from the eggs deposited by the flies in this cage. The same is true for the first counts in cages Nos. 45–49 recorded in tables 4–6.

a level close to 80 percent ST also appears to be established. The adaptive values and selection coefficients deduced from data in table 5 are as follows:

	Genotype	*W*	
Cage No. 46	ST^P/ST^P	0.93	s=0.07
	ST^P/AR^M	1.00	t=0.40
	AR^M/AR^M	0.60	
Cage No. 47	ST^M/ST^M	0.88	s=0.12
	ST^M/AR^P	1.00	t=0.63
	AR^P/AR^P	0.37	

In both cages the adaptive values are ST/AR>ST/ST>AR/AR, in other words, the reverse of the viability relations in cage No. 46 (see above). What is the basis of this inconsistency, which is merely an extreme case of the relatively minor disagreements between the estimates of the adaptive values and the viability coefficients for cages Nos. 45, 48, and 49?

The answer is that the relative viability under competition is only one of the variables which determine the adaptive value of a genotype. Our estimates of the viability describe the survival rates of the carriers of different chromosomal types between the time when zygotes are formed and the hatching of the adult from pupae. These rates need not necessarily be, and evidently are not, either quantitatively or qualitatively consonant with the duration of life of the adult, fecundity, sexual activity, and other variables which influence the adaptive value. And it is the adaptive value which determines the selective fate of a genotype.

The discrepancy here observed is not unique. WALLACE (1948) found that larvae of *Drosophila pseudoobscura* heterozygous for the "sex-ratio" condition in the X-chromosome are superior in viability to homozygous normal larvae, and these are superior to homozygous "sex-ratio" larvae. Yet, the adult longevity is the same in heterozygotes and in normal homozygotes, while "sex-ratio" homozygotes are much inferior. The normal and "sex-ratio" homozygotes are equal to each other in fecundity but inferior to heterozygotes. Similar lack of concordance in different traits was found by HEUTS (1948) in homozygotes and heterozygotes for different gene arrangements in the third chromosome of the Piñon Flats populations of *D. pseudoobscura*. In *D. polymorpha* homozygotes and heterozygotes for a certain gene can be distinguished by the color of the abdomen. DA CUNHA (1949) found in natural populations of this species deviations from the ratios demanded by the binomial square rule in favor of homozygotes, while in artificial populations of the same species the deviations are in favor of the heterozygotes. Adaptive differences between variants of a species are often so complex that superiority in one trait often goes with inferiority in other traits. The adaptive value of a variant is the result of interaction of numerous variables.

DISCUSSION

Although hybrid vigor, or heterosis, has been studied for a long time, only in recent years has it become evident that these terms (which are usually

treated as synonymous) are common names for a group of scarcely related phenomena (DOBZHANSKY 1947b, 1949; CROW 1948). The situation in *Drosophila pseudoobscura* is instructive because in this species two different kinds of heterosis are reasonably well known and understood. The first kind arises from the presence in populations of deleterious recessive mutant genes sheltered by their normal dominant alleles. Accumulation of these deleterious genes is a by-product of the mutation process. The second kind of heterosis is due to complexes of linked polygenes which give specific "heterotic" interaction effects in heterozygotes ("overdominance," cf. HULL 1946). This kind of heterosis is engendered by natural selection as a form of adaptation of the species to its environment.

Mutations that arise in any species are usually deleterious to their carriers. Now, deleterious dominant and semi-dominant mutants are eliminated relatively rapidly by natural selection; recessive and near recessive mutants accumulate in populations of outbreeding species. A majority of individuals in natural populations of *D. pseudoobscura* carry one or more deleterious recessive mutants in heterozygous condition (DOBZHANSKY, HOLZ, and SPASSKY 1942). Inbreeding makes the deleterious recessives homozygous, which results in loss of fitness in the inbred line. Intercrossing of inbred lines results in restoration of the normal level of vigor. This is not because dominant gene alleles are intrinsically more favorable than recessive ones, but because dominant alleles present in a population are rigidly controlled by natural selection.

Since physical and biotic environments are constant neither in space nor in time, a living species meets a variety of environments. It responds to the challenge of variable environments by evolving genetically controlled adaptive polymorphism. One of the forms of adaptive polymorphism is balanced polymorphism. Suppose that a population contains alternative genetic variants A^1, A^2, and A^3, and that the heterozygotes A^1A^2, A^1A^3, and A^2A^3 are heterotic, i.e., have higher net fitness (adaptive value) than the homozygotes A^1A^1, A^2A^2, and A^3A^3. The variants A^1, A^2, and A^3 may be gene alleles, linked gene complexes, or chromosomal variants, such as gene arrangements modified by inversions or translocations. Natural selection will preserve all heterotic genetic variants, even if the adaptive values of the homozygotes are very low. The greater the number of balanced alternative variants, the greater the proportion in an outbred population of highly fit heterozygotes, and the lower the frequency of the less well adapted homozygotes. It is, of course, possible that the heterozygotes may be adapted for occupation of ecological niches which are widespread in natural habitats, and the homozygotes may occupy more restricted habitats. Natural selection favors attainment of a high level of adaptedness by the population as a whole, even at the expense of producing some inferior or narrowly specialized types. Balanced polymorphism enables an outbreeding species to attain high mean fitness, and at the same time to preserve great evolutionary plasticity. To be sure, balanced polymorphism is not incompatible with some inbreeding. By a kind of genetical *tour de force*, some species of Oenothera have evolved mechanisms which combine heterosis with virtually obligatory self-pollination. Nevertheless, inbreeding in a nor-

mally crossbred species usually leads to loss of fitness, and crossing of inbred strains, to restoration of normal reproductive biology and of the normal heterotic state.

In Drosophila, the variations in the gene arrangement in chromosomes of natural populations are often, and perhaps always, connected with the maintenance of heterosis. The adaptive superiority of inversion heterozygotes to homozygotes in *Drosophila pseudoobscura* has been established by DOBZHANSKY (1943, 1947 a, b, 1949), WRIGHT and DOBZHANSKY (1946), DOBZHANSKY and LEVENE (1948), and WALLACE (1948). DUBININ and TINIAKOV (1945, 1946 a, b) demonstrated the adaptive nature of the inversions in *D. funebris*, SPIESS (1950) in *D. persimilis*, CARSON and STALKER (1947) and LEVITAN (unpublished) in *D. robusta*. The only case of balanced polymorphism in Drosophila not known to be connected with chromosomal inversions is that studied by *da Cunha* (1949) in *D. polymorpha*, a species unfavorable for cytological study.

In *D. pseudoobscura*, inversion heterozygotes which carry two chromosomes derived from the same population usually show heterosis, while heterozygotes which carry chromosomes from geographically remote populations usually do not. Heterosis is here the outcome of interaction of polygene complexes borne in the chromosomes with different gene arrangements and protected by the inversions from disintegration by crossing over in the heterozygotes. The mutual adjustment, or coadaptation, of the polygene complexes which occur in the population of a geographical region is clearly an outcome of evolutionary development controlled by natural selection. Natural selection is responsible for the origin of heterosis as well as for its maintenance. CROW (1948) has presented arguments to show that heterosis in *Zea mays* is caused largely by interaction of genes, or gene complexes, which are deleterious when homozygous, *i.e.*, by the second of the two mechanisms described above for *D. pseudoobscura*.

Other types of "heterosis" have also been recorded in the literature. The term "heterosis" is sometimes applied to situations in which hybrids show "the increased size, the excessive kinetic energy, the increased productiveness," but no evidence of increased adaptive value compared to the parental forms (SHULL 1948). It has been shown above that the adaptive values of the chromosomal types of *D. pseudoobscura* are not necessarily parallel to the relative viabilities of these types between the egg and the adult stage. It is naive to assume, as is so often implied in discussions of heterosis, that a hybrid which is larger or more rapidly growing than its parents is also more "vigorous." Surely, there must be some optimal size of the body, of its parts, optimal fecundity, and optimal rates of growth and of metabolic processes for every species. Increases above the optima result, then, in losses rather than in gains of adaptive value.

Cases were already known in the pre-Mendelian period when artificially obtained hybrids between normally outbred species exhibited "luxuriance" in size or in growth rate. It is sometimes asserted that the mule is "heterotic" compared to its parents, the horse and the ass. Artificial hybrids between normally self-pollinating and sympatric strains of certain plant species have

also been called "heterotic" because of their larger size. It is doubtful, or at least uncertain, whether these enlarged hybrids would show adaptive values superior to their parents and prove more vigorous in competition with the latter in natural habitats. The advisability of applying the term "heterosis" to cases in which heterozygotes are larger in body size, or show "increases" in any "traits," but no evidence of higher adaptive value compared to the corresponding homozygotes, is open to question. Perhaps the word "luxuriance" would be a better designation for such cases, the word "heterosis" or "euheterosis" to be used for adaptive superiority of heterozygotes to homozygotes. Whichever way the terminological problem is decided, it is clear that the mechanisms underlying euheterosis and luxuriance are quite different. Euheterosis is a product of mutation and selection pressures on populations of outbreeding species. Luxuriance is a result of gene interactions which are not causally related to the reproductive biology of the species.

Euheterosis and luxuriance occur, of course, both in wild and in domesticated forms. In the latter, euheterosis is brought about by combined action of artificial and natural selection. Just as natural selection engenders and maintains gene complexes which confer adaptive superiority on heterozygotes in outbreeding wild species, in outbred cultivated species, artificial selection establishes gene complexes which lead the heterozygotes to possess qualities desired by man. Similarly, artificial selection eliminates the unfavorable dominants and semidominants more rapidly than the unfavorable recessives. Euheterosis is always a form of evolutionary adaptation—to the environment in wild species and to the human needs or desires in domesticated ones. Luxuriance is in either case an evolutionary accident.

SUMMARY

Inversion heterozygotes in *Drosophila pseudoobscura* which carry two chromosomes derived from the same population are, as a rule, superior in adaptive value to the homozygotes. In contrast to this, inversion heterozygotes which carry two chromosomes of different geographic origin usually show no heterosis. In one of the experiments, in which the chromosomes came from remote localities (California and Mexico), the heterozygotes were even inferior to both homozygotes. The heterosis is produced, in these cases, by interaction of polygene complexes, which have become mutually adjusted, or coadapted, by natural selection in the course of the evolutionary process. Polygene complexes which occur in geographically distant localities have not undergone the process of coadaptation.

The adaptive value (net fitness) of a type is not necessarily proportional to its survival value at all developmental stages. In our experiments, some types which showed relatively higher mortalities than other types between the egg and the adult stage proved nevertheless to be adaptively superior to the latter. The adaptive values are determined, in our experiments, by observing the changes in the relative frequencies of the two types with time in populations breeding in population cages.

The causation of different forms of heterosis is discussed. Heterosis proper, or *euheterosis*, is distinguished from *luxuriance* of heterozygotes. The former is

a product of mutation and selection pressures; the latter is an evolutionary accident.

ACKNOWLEDGMENT

The writer wishes to acknowledge his obligation to DOCTOR HOWARD LEVENE for his advice regarding the statistical treatment of the data and, in particular, for the formulae used in calculation of the survival values. The selection coefficients and the adaptive values were calculated under the direction of PROFESSOR SEWALL WRIGHT. MISS CHARLOTTE MADISON, MRS. E. SCHEREMETJEVA-BRUNST, and MESSRS. M. FOSTER, B. SPASSKY, and S. ZIMMERING assisted in conducting the experiments, making the cytological preparations, and carrying out most of the calculations. All the classifications of the gene arrangements were, however, made by the writer. The ROCKEFELLER FOUNDATION and the CARNEGIE INSTITUTION OF WASHINGTON provided financial support for the work.

LITERATURE CITED

CARSON, H. L., and H. D. STALKER, 1947 Gene arrangements in natural populations of *Drosophila rubusta* Sturtevant. Evolution **1**: 113–133.

CROW, J. F., 1948 Alternative hypotheses of hybrid vigor. Genetics **33**: 477–487.

CUNHA, A. BRITO DA, 1949 Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. Evolution **3**: 239–251.

DOBZHANSKY, TH., 1943 Genetics of natural populations. IX. Genetics **28**: 162–186.

1947a Genetics of natural populations. XIV. Genetics **32**: 142–160.

1947b Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution **1**: 1–16.

1948a Genetics of natural populations. XVI. Genetics **33**: 158–176.

1948b Genetics of natural populations. XVIII. Genetics **33**: 588–602.

1949 Observations and experiments on natural selection in Drosophila. Proc. 8th Int. Cong. Genetics: 210–224.

DOBZHANSKY, TH., and C. EPLING, 1948 The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. nat. Acad. Sci. **34**: 137–141.

DOBZHANSKY, TH., A. M. HOLZ, and B. SPASSKY, 1942 Genetics of natural populations. VIII. Genetics **27**: 463–490.

DOBZHANSKY, TH., and H. LEVENE, 1948 Genetics of natural populations. XVII. Genetics **33**: 537–547.

DUBININ, N. P., and G. G. TINIAKOV, 1945 Seasonal cycles and the concentration of inversions in populations of *Drosophila funebris*. Amer. Nat. **79**: 570–572.

1946a Natural selection and chromosomal variability in populations of *Drosophila funebris*. J. Hered. **37**: 39–44.

1946b Inversion gradients and natural selection in ecological races of *Drosophila funebris*. Genetics **31**: 537–545.

HEUTS, M. J., 1948 Adaptive properties of carriers of certain gene arrangements in *Drosophila pseudoobscura*. Heredity **2**: 63–75.

HULL, F. H., 1946 Overdominance and corn breeding where hybrid seed is not feasible. J. Amer. Soc. Agron. **38**: 1100–1103.

SHULL, G. H., 1948 What is "heterosis"? Genetics **33**: 439–446.

SPIESS, E., 1950 Experimental populations of Drosophila persimilis from an altitudinal transect of the Sierra Nevada. Evolution **4**: 14–33.

WALLACE, B., 1948 Studies on "Sex-Ratio" in *Drosophila pseudoobscura*. I. Evolution **2**: 189–217.

WRIGHT, S., and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Genetics **31**: 125–156.

ANALYSIS OF QUANTITATIVE GENE ACTION BY CONSTANT PARENT REGRESSION AND RELATED TECHNIQUES[1]

BRUCE GRIFFING
Iowa State College, Ames, Iowa

Received November 14, 1949

ANALYSIS of multigenic or quantitative inheritance is one of the important problems of theoretical and applied genetics. Such analysis requires techniques of a different nature from those used in studying individual genes of large effect. The techniques thus far devised have not been wholly satisfactory, and for this reason new methods of analyzing quantitative inheritance are of considerable interest. A relatively new method, the constant parent regression technique, is reviewed and extended in this paper.

There is a considerable body of evidence to demonstrate that typical quantitative characters are controlled by a large number of gene pairs. It is assumed that the following statements are corollaries of this multigenic nature of inheritance: (1) linkage results because the large number of quantitative genes for a particular trait are located on a relatively small number of chromosome pairs, and (2) in general, the effect of each gene pair is relatively small. Because of this low order of effect of individual genes, it is necessary to study the action of these genes "en masse" by statistical techniques. This obviously results in inferences about the average properties of a set of quantitative genes.

To estimate the average gene action one may construct models involving different types of gene interaction and choose the model which best fits the experimental data. In thus reducing the total gene action entering into the expression of a complex characteristic to that of a simple model, it cannot be assumed that all genes behave in the manner prescribed by the chosen model. In fact, it has been suggested that quantitative genes probably have as diverse types of action as the so-called qualitative ones and differ from qualitative genes only in magnitude of effect. However, it has been shown in various quantitative traits that the system of genes involved does have average properties which are measurable. Estimation of these group genetic parameters is the objective of a statistical analysis, and these estimated parameters are associated with a gene model. The principal approach that has developed over the years involves, (1) a description of frequency distributions resulting from segregating populations by use of first, second, and third moments, and (2) a partitioning of variances into components. Most of the techniques have been summarized by MATHER (1949).

Use of segregating populations offers certain advantages but entails both theoretical and practical difficulties. First, with segregation, linkage effects are generated which contribute to the genotypic variance. This complex problem has been dealt with to some extent by MATHER (1949). Second, a large

[1] Journal Paper No. J-1719 of the IOWA AGRICULTURAL EXPERIMENT STATION, Ames, Iowa. Project No. 250.

number of individuals are needed to estimate properly the parameters of any one particular genetically segregating population. For example: justifiably to partition the variance of an F_2, the experimenter needs to include a fairly large number of F_3 or biparental families each with adequate number of individuals to estimate their own distributions. This leads to the physical difficulty of making observations on a vast number of individuals. If the characteristics involved are functions of indeterminate type of growth, such as yield of tomatoes, the amount of work becomes tremendous. Third, difficulties arise in designing experiments from which estimates of different populations are accurate and comparable. There is also the problem of confounding genetic and block effects in a replicated experiment.

By considering only the non-segregating generations (homozygous parents and F_1s) the constant parent regression method, evolved by Hull (1946, 1947), considerably reduces the previously mentioned sources of error. Since the P_1s and F_1s are not segregating, relatively few plants are needed to estimate the expected genotypic values. The actual number necessary depends on the heritability of the traits involved, and the accuracy may be increased to any practical desired level by merely increasing the number of plants. Variance within a line is assumed to be entirely environmental and thus, with appropriate sampling techniques, an analysis of the source of these environmental variations is provided. Experimental designing presents no problems and there is no genetic confounding. (There is, of course, a genotypic-environmental interaction which may be estimated.)

Linkage disturbs the constant parent regression analysis only in the sampling procedure of obtaining the set of parents used from all theoretical possible homozygous genotypes. In populations resulting from recent hybrid origin, linkages existing in the original parental types may reduce the probability of occurrence of some gene combinations and raise the probability of obtaining others. It can be demonstrated that coupling linkages do not affect the constant parent regression results whereas repulsion linkages may give rise to some pseudo-overdominance as measured by the second order regression coefficient. However, with random mating over a period of time the coupling and repulsion phases are expected to become equally frequent in the population, and thus, the effect of linkage in sampling parental lines disappears. The possibility of linkage effect should not be overlooked but in most cases is probably of little concern.

Experiments with segregating populations generally involve only two original parents. On the other hand, the P_1-F_1 test is conducted with a variety of different parents, thus providing a wider sampling of the available germplasm, and allowing broader inferences to be made from the result obtained. By using a number of parents, the experimenter interested in more than one characteristic should be able to choose lines which collectively give desired ranges of expression in all traits. This might be difficult to do when only two parents must be chosen.

The P_1-F_1 methodology also directly and easily yields estimates of herita-

bility and of phenotypic and genotypic correlations. These estimates may be used to develop discriminant function selection indices if desired (see GRIFFING 1948).

The main disadvantages of the P_1-F_1 technique are that estimation of gene number or number of effective factors (MATHER 1949), detection of major genes and examination of gene-chromosome relationships which could involve linkage and phase of linkage are not directly possible.

CONSTANT PARENT REGRESSION METHOD

The following presentation will consider the experimental situation in which a set of inbred (homozygous) lines are available together with all possible F_1 combinations of these lines. These restrictions are not entirely necessary in that not all F_1s need be present, different males and females may be used, and F_2s and later generations may be considered (HULL 1947a). However, for simplicity the above mentioned restrictions will be imposed. Generally, one set of F_1s is used and not the reciprocals, *i.e.* $F_{ij} = F_{ji}$ in table 1.

Notation is as follows:

P_i = i^{th} parent
F_{ij} = F_1 (or hybrid) of i^{th} and j^{th} parents
$F_{ij} = F_{ji}$
c.p. = constant parent (homozygous)
c.p.r. = constant parent regression, also noted as bp.

F_1 and F_{ij} are used interchangeably. The term "F_1" is used in the general sense, and F_{ij} is used more specifically to indicate the F_1 of the i^{th} and j^{th} parents.

(1) "n" parents are crossed in all possible combinations of pairs to give

$$\binom{n}{2} = \frac{n!}{2!(n-2)!} \text{ hybrids.}$$

TABLE 1

Combination of n inbred lines to give all possible F_1's.

	P_1	P_2	P_3 - - - - - - - - - - P_j - - - - P_n
P_1	P_1	F_{12}	F_{13} - - - - - - - - - - F_{1j} - - - - F_{1n}
P_2	F_{21}	P_2	F_{23} - - - - - - - - - - F_{2j} - - - - F_{2n}
P_3	F_{31}	F_{32}	P_3 - - - - - - - - - - F_{3j} - - - - F_{3n}
.	.	.	. . .
.	.	.	. . .
.	.	.	. . .
P_i	F_{i1}	F_{i2}	F_{i3} - - - - P_i - - - - F_{ij} - - - - F_{in}
.	.	.	. . . .
.	.	.	. . . .
.	.	.	. . . .
P_n	F_{n1}	F_{n2}	F_{n3} - - - - F_{ni} - - - - F_{nj} - - - - P_n

(2) Since there are "n" parents there are "n" c.p. groups; the i^{th} c.p. group is defined as follows:
   (a) The i^{th} row constitutes the i^{th} c.p. group and has
      1. P_i as c.p.; P_j where $j=1, 2, \cdots, n, i \neq j$ as variable parents.
      2. $(n-1)$ hybrids ($F_{i1}, F_{i2}, \cdots, F_{in}$); *i.e.*, F_1s resulting from P_i crossed with all other parents.

With this scheme as a basis, HULL (1946) ingeniously devised a statistical approach to the problem of estimating the relative dominance effect for the simplest gene model in which between-loci-effects are additive (no between-loci-interactions). We shall consider this gene model as well as some aspects of an epistatic gene model in which between-loci interactions occur.

The statistical approach is made by evaluating the trend of c.p.r. coefficients. This involves a regression of F_1s on the variable parents within each c.p. group. A c.p.r. coefficient is calculated for each c.p. group. As will be shown later, the trend (increasing or decreasing) of c.p.r. coefficients relative to the value of the corresponding c.p. will give information regarding both direction and magnitude of dominance. This trend is measured by a "second order" regression, *i.e.*, the regression of c.p.r.'s on the c.p. values.

First, models involving only two-gene pairs will be considered, and later these will be extended to the "n" gene case, using models having three-gene pairs to point out difficulties encountered.

A. *Two-gene-pair models*

With two gene pairs, four c.p. groups are possible, each having three F_1s, as shown in table 2.

Two types of models will be discussed, one involving dominance alone with no epistasis and the other considering both dominance and epistasis.

1. Dominance but no epistasis

Assumptions:

1. Gene effects are the same for both gene pairs. The notation followed is a modification of FISHER (1918).

$A_1A_1=A_2A_2=2d$	where:	$h=0$	no dominance
		$h=\pm 1$	complete dominance
$A_1a_1=A_2a_2=d+dh$		$-1<h<0$	incomplete neg. dom.
		$0<h<+1$	incomplete pos. dom.
$a_1a_1=a_2a_2=0$		$h>+1$ or $h<-1$	super or over dom.

2. Between-loci effects are additive.

These algebraic values give rise to table 3.

If c.p.r. coefficients are calculated for each c.p. group, one will find that they take on the algebraic values found in table 4.

On examination of table 4, it is obvious that the direction of c.p.r. trend, with respect to parental values, indicates plus or minus dominance. Thus, with no dominance ($h=0$) there is no trend; all c.p.r. coefficients are equal to .5. Diagrammatically, all the F_1s lie at midparental values, and, considering values with no error, there will be no deviations from regression. With positive

TABLE 2

General arrangement of Ps and all possible Fis for two-gene models.

	P_1	P_2	P_3	P_4
	$a_1a_1a_2a_2$	$A_1A_1a_2a_2$	$a_1a_1A_2A_2$	$A_1A_1A_2A_2$
P_1	P_1	$A_1a_1a_2a_2$	$a_1a_1A_2a_2$	$A_1a_1A_2a_2$
P_2		P_2	$A_1a_1A_2a_2$	$A_1A_1A_2a_2$
P_3			P_3	$A_1a_1A_2A_2$
P_4				P_4

dominance a decreasing trend occurs for the c.p.r. coefficients as the parental values increase. This trend becomes more severe with an increase of the dominance value. With complete positive dominance the trend goes from +1.0 to 0, and with overdominance the extreme c.p.r. values are 1+a/2 and −a/2. In this way a c.p.r. exceeding one or less than zero is indicative of overdominance. The c.p.r. values together with their trend as measured by b_2 give an indirect method of evaluating the direction and magnitude of dominance. A test of significance for b_2 and a more direct method of estimating "h" will be discussed later.

TABLE 3

Algebraic values for Pis and Fis for a two-gene model.

		P_1 0	P_2 2d	P_3 2d	P_4 4d
P_1	0		d+dh	d+dh	2d+2dh
P_2	2d	d+dh		2d+2dh	3d+dh
P_3	2d	d+dh	2d+2dh		3d+dh
P_4	4d	2d+2dh	3d+dh	3d+dh	

TABLE 4

Algebraic values for c.p.r.'s, together with values for these regression coefficients with different values of "h."

CONSTANT PARENT	CONSTANT PARENT REGRESSION VALUES					
	ALGEBRAIC VALUES	h=0	h=+1	h>+1 OR h=1+a, a>0	h=−1	n<−1 OR h=a−1, a<0
$P_1=0$	$\frac{1+h}{2}$	.5	1.0	$1+\frac{a}{2}$	0	$+\frac{a}{2}$
$P_2=2d$	.5	.5	.5	.5	.5	.5
$P_3=2d$	.5	.5	.5	.5	.5	.5
$P_4=4d$	$\frac{1-h}{2}$	.5	0	$-\frac{a}{2}$	1.0	$1-\frac{a}{2}$

$b_2=-\frac{h}{4d}$ (second order regression coeff. of c.p.r. on c.p. value)

2. Both dominance and epistasis

In setting up this model we may regard diagrammatically epistasis as an interaction between loci analogous to dominance as an interaction between alleles at the same locus. The basic diagram is the following which shows the four parental values.

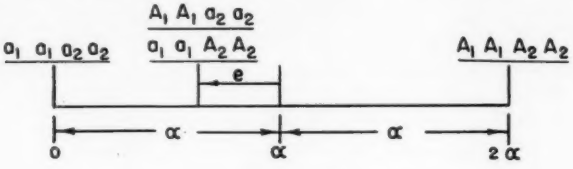


DIAGRAM 1

The genotypic values for the heterozygotes may be obtained by the fact that the F_1 is a dominance deviation away from the midpoint between its homozygotes. For example, the following diagram shows the derivation of $A_1a_1a_2a_2$.

$$A_1a_1a_2a_2 = 1/2(1 - e)(1 + h)\alpha.$$

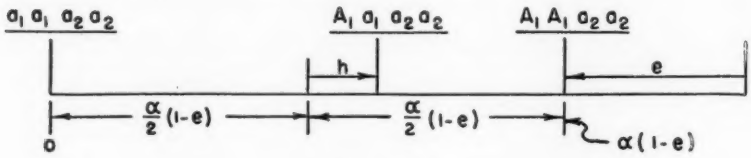


DIAGRAM 2

Following such a scheme all P_1 and F_1 values may be established, and the algebraic values are given in table 5.

TABLE 5

Algebraic values for P_1s and F_1s involving both dominance and epistasis.

	P_1 $\frac{a_1a_1a_2a_2}{0}$	P_2 $\frac{A_1A_1a_2a_2}{\alpha(1-e)}$	P_3 $\frac{a_1a_1A_2A_2}{\alpha(1-e)}$	P_4 $\frac{A_1A_1A_2A_2}{2\alpha}$
$P_1=0$		$\frac{\alpha}{2}(1-e+h-eh)$	$\frac{\alpha}{2}(1-e+h-eh)$	$\frac{\alpha}{2}(2-e+2h+eh^2)$
$P_2=\alpha(1-e)$	$\frac{\alpha}{2}(1-e+h-eh)$		$\frac{\alpha}{2}(2-e+2h+eh^2)$	$\frac{\alpha}{2}(3-e+h+eh)$
$P_3=\alpha(1-e)$	$\frac{\alpha}{2}(1-e+h-eh)$	$\frac{\alpha}{2}(2-e+2h+eh^2)$		$\frac{\alpha}{2}(3-e+h+eh)$
$P_4=2\alpha$	$\frac{\alpha}{2}(2-e+2h+eh^2)$	$\frac{\alpha}{2}(3-e+h+eh)$	$\frac{\alpha}{2}(3-e+h+eh)$	

TABLE 6

Algebraic values for c.p.r. coefficients for both dominance and epistasis.

CONSTANT PARENT	C.P.R. COEFFICIENTS
$P_1=0$	$bp_1=\frac{1}{2}\ \frac{\{(1+h)+e[(1+h)^2+eh(1+h)]\}}{(1+e)^2}$
$P_2=\alpha(1-e)$	$bp_2=\frac{9-2eh(2eh-3)}{6(e^2+3)}$
$P_3=\alpha(1-e)$	$bp_3=\frac{9-2eh(2eh-3)}{6(e^2+3)}$
$P_4=2\alpha$	$bp_4=\frac{1}{2}\ \frac{\{(1-h)-e[(1-h)^2+eh(1-h)]\}}{(1-e)^2}$

Using these algebraic values, c.p.r. coefficients may be calculated for each c.p. group, and these are listed in table 6.

It may be noted from tables 5 and 6 that if "e" is equated to zero, then all algebraic values reduce to those in the first model (letting $\alpha=2d$).

A two-way classification table may be constructed to demonstrate the trends of c.p.r. coefficients for different numerical values of "e" and "h." These values are found in table 7.

The following characteristics of the "e" by "h" table may be noted.

(1) When $e=0$, $h=0$, $bp_i=.5$, as found earlier.

(2) When $e=0$, and "h" varied (first row), the same trend occurs as found in table 4, which is a steadily decreasing regression trend from bp_1 to bp_4. The relative decrease depends on the degree of dominance.

TABLE 7

Two-way classification of various values of "e" and "h" for constant parent regression coefficients.

EPISTASIS	DOMINANCE		
	h= 0	h=+.5	h=+1.0
e=0	$bp_1=$ .50	= .75	= 1.00
	$bp_2=$ .50	= .50	= .50
	$bp_3=$ .50	= .50	= .50
	$bp_4=$ .50	= .25	= 0
e=+.5	$bp_1=$ .33	= .63	= 1.00
	$bp_2=$ .46	= .53	= .56
	$bp_3=$ .46	= .53	= .56
	$bp_4=$1.00	= .63	= 0
e=+1.0	$bp_1=$ .25	= .56	= 1.00
	$bp_2=$ .38	= .46	= .46
	$bp_3=$ .38	= .46	= .46
	$bp_4=+\infty$	$=+\infty$	$=+\infty$

(3) When h=0, and "e" varied (first column), epistasis is considered alone without the confounding influence of dominance. The regression trend is opposite to that of dominance alone, as the regression trend steadily increases from bp_1 to bp_4. The relative increase depends on the degree of epistasis.

(4) When "h" and "e" both have values other than zero—*i.e.*, e=+.5, h=+.5—then conflicting regression trends result in a decrease from bp_1 to bp_2 and an increase from bp_3 to bp_4.

A consideration of RASMUSSON's (1933) interaction hypothesis can be made by merely assigning "e" negative values. The hypothesis may be summarized by stating that the phenotypic result of the addition of genes follows the law of diminishing returns. In other words, genes added when the character expression is low would have much greater effect than when the character expression is near a physiological limit.

The above two-gene pair model is only one of many possible approaches to between-loci interaction. It is an attempt to combine in one model both dominance and epistatic effects and still allow algebraic solution. An extension of this model to more gene pairs may be difficult and for an extended epistatic and dominance model the logarithmic case is chosen and will be discussed later.

HULL (1947a) has presented a regression equation which involves both dominance and complementary gene action.

BURDICK (1949) has developed an epistatic model for multiplicative effects of gene pairs assuming no dominance. The c.p.r.s yield an increasing trend in much the same manner as the epistatic model described above with h=0.

Testing for significance of b_2 (in both models) by an ordinary analysis of variance for the regression of c.p.r. coefficients on parental values is not valid because the c.p.r. coefficients are highly correlated. The problem can be approached by fitting constants to the following regression equation by least squares procedure:

$$F_{ij} = m + a_1(P_i + P_j) + a_2(P_i^2 + P_j^2) + a_3(P_iP_j) + e_{ij}$$

i.e. by estimating and testing a_1, a_2, and a_3. Because a_3 is equal to b_2 for the case of dominance alone, a test of significance of a_3 is equivalent to a test of significance for b_2 for dominance alone. Similarly since a_2 approximates b_2 for the case of epistasis alone (for the two-gene-pair model) a test of significance for a_2 is equivalent to a test for b_2 for epistasis alone. When "e" and "h" both have some value, the c.p.r. trend is curvilinear with respect to c.p. values so that the regression coefficient b_2 would have little meaning.

B. *Extension of two-gene-pair models*

1. Dominance but no epistasis

Model specifications are as follows:

1. There are "n" loci affecting the specified character.
2. Effect of gene pairs are identical. However, both d and h may vary.

$$A_1A_1 = A_2A_2 \cdots = A_nA_n = 2d$$

$$A_1a_1 = A_2a_2 \cdots = A_na_n = d + dh$$

$$a_1a_1 = a_2a_2 \cdots = a_na_n = 0.$$

3. Effects of genes at different loci are additive.

With "n" loci and the alternative of two alleles at any one locus, there are 2^n number of different possible homozygous parental lines. These inbreds may be denoted simply by their gamete arrangement, and all possible gamete arrangements may be indicated merely by the expansion of

$$\prod_{i=1}^{n} (A_i + a_i).$$

Parents of different genotypic constitution may have the same genotypic value, and thus F_1s with different genotypic values may result from crosses in which the parents have the same genotypic values. For example, from the three-gene-pair case as illustrated in table 8, one can see that parent No. 6 ($A_1a_2A_3$)

TABLE 8

Algebraic values for all possible parents and F_1s involved in the three-gene-pair model.

			P_1 $a_1a_2a_3$ 0	P_2 $A_1a_2a_3$ 2d	P_3 $a_1A_2a_3$ 2d	P_4 $a_1a_2A_3$ 2d	P_5 $A_1A_2a_3$ 4d	P_6 $A_1a_2A_3$ 4d	P_7 $a_1A_2A_3$ 4d	P_8 $A_1A_2A_3$ 6d
P_1	$a_1a_2a_3$	0	0	d+dh	d+dh	d+dh	2d+2dh	2d+2dh	2d+2dh	3d+3dh
P_2	$A_1a_2a_3$	2d	d+dh	2d	2d+2dh	2d+2dh	3d+dh	3d+dh	3d+3dh	4d+2dh
P_3	$a_1A_2a_3$	2d	d+dh	2d+2dh	2d	2d+2dh	3d+dh	3d+3dh	3d+dh	4d+2dh
P_4	$a_1a_2A_3$	2d	d+dh	2d+2dh	2d+2dh	2d	3d+3dh	3d+dh	3d+dh	4d+2dh
P_5	$A_1A_2a_3$	4d	2d+2dh	3d+dh	3d+dh	3d+3dh	4d	4d+2dh	4d+2dh	5d+dh
P_6	$A_1a_2A_3$	4d	2d+2dh	3d+dh	3d+3dh	3d+dh	4d+2dh	4d	4d+2dh	5d+dh
P_7	$a_1A_2A_3$	4d	2d+2dh	3d+3dh	3d+dh	3d+dh	4d+2dh	4d+2dh	4d	5d+dh
P_8	$A_1A_2A_3$	6d	3d+3dh	4d+2dh	4d+2dh	4d+2dh	5d+dh	5d+dh	5d+dh	6d

has a value of 4d, and parents No. 4 ($A_1a_2a_3$) and No. 3 ($a_1A_2a_3$) both have values of 2d. However, the F_1 (6×4) = 3d+dh, and the F_1 (6×3) has a different value namely, 3d+3dh. Therefore, it is impossible to determine uniquely the F_1 genotypic value with knowledge of only the parental genotypic values. In other words, in the three-dimensional coordinate system, where P_i, P_j and F_{ij} are the three axes, a three-dimensional constellation of points would be generated with "n" gene pairs rather than a simple surface (if the measurements used were the genotypic values).

HULL (1946, 1947) suggests a condensation process, which may be illustrated by the three-gene pair example, for collapsing the scatter of F_1s into a regression surface. In table 8, all rows having parents of like genotypic values are combined and similarly all columns of like genotypic values are combined. Table 8 then reduces to table 9, which has only one row and one column for

TABLE 9

Algebraic values for the condensed table involving the three-gene-pair model.

		P_1	P_2	P_3	P_4
		0	2d	4d	6d
P_1	0	0	d+dh	2d+2dh	3d+3dh
P_2	2d	d+dh	$2d+\frac{4}{3}dh$	$3d+\frac{5}{3}dh$	4d+2dh
P_3	4d	2d+2dh	$3d+\frac{5}{3}dh$	$4d+\frac{4}{3}dh$	4d+dh
P_4	6d	3d+3dh	4d+2dh	5d+dh	6d

each parental genotypic value. The F_1 values in table 9 are averages and may be obtained in two ways.

1. The F_1 values in table 8 may be averaged in the manner described above.
2. HULL (1946, 1947) suggests the following general scheme for determining the genotypic value for any particular F_1 cell of table 9.

Let u and w be the proportion of AA loci in P_i and P_j, respectively, and $(1-u)$ and $(1-w)$ be the proportion of aa loci. The average F_1 value may be determined as follows:

			P_i gametes	
			A	a
			u	$1-u$
	A	w	uw	$w(1-u)$
P_j				
gametes				
	a	$1-w$	$u(1-w)$	$(1-u)(1-w)$

Parental coded values are:

$P_i = u$ 2nd

$P_j = w$ 2nd

Then

$$F_{ij} = uw(2nd) + \{w(1-u) + u(1-w)\}n(d + dh). \qquad (1)$$

(Parental and F_1 values are coded so that the completely recessive genotype, $a_1a_1a_2a_2 \cdots a_na_n$, has a genotypic value of zero.)

With HULL's theoretical procedure a regression surface is fitted to the three-dimensional scatter, and equation (1) may be used to predict the exact average F_1 values found in the cells of table 9.

Let us examine the situation more closely by considering separately the four constant parent groups possible from table 9. In figure 1, each constant parent group is plotted together with the regression of the F_1s on the variable parents. In all cases the F_1 values fall directly on the regression line, and the regression coefficients form a linear series so that there is a perfect, simple correlation between regression coefficients and the corresponding constant parent value.

To illustrate this point, one may first re-write equation (1) in terms of parental values and then partially differentiate the F_1 with respect to the variable parent.

$$F_{ij} = \frac{P_i + P_j}{2}(1 + h) - \frac{h}{2nd} P_i P_j.$$

The rate of change in the F_{ij} with change in P_j (variable parent) and considering P_i as the constant parent may be expressed as follows:

$$\frac{\partial F_{ij}}{\partial P_j} = \frac{1 + h}{2} - \frac{h}{2nd} P_i = bp_i \text{ (c.p.r. for } P_i).$$

The second order regression coefficient, which represents the rate of change in bp_i with change in the constant parent (P_i), may be represented as follows:

$$\frac{\partial bp_i}{\partial P_i} = -\frac{h}{2nd} = b_2.$$

However, in practice where, obviously, only a sample of all possible parental genotypes can be used one is not confronted with the idealized regression surface but rather the actual F_1 scatter. Under such conditions the c.p.r. scheme is not exact. This may be illustrated by forming different sets of parents in

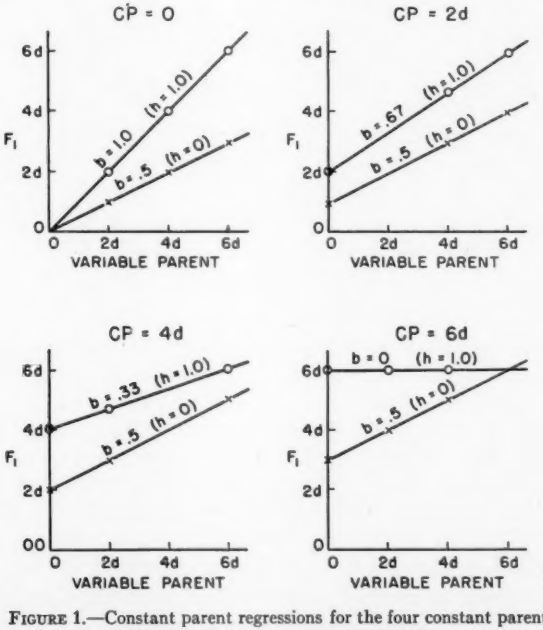
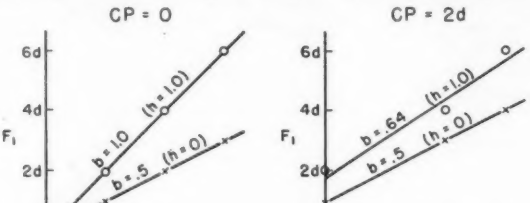


FIGURE 1.—Constant parent regressions for the four constant parent groups involved in the condensed set of F_1s.

which no one set contains all possible parents. From the three-gene-model, let us consider two parental sets ($S_1 = P_1$, P_2, P_5, P_8 and $S_2 = P_1$, P_4, P_5, P_8) chosen from table 8. Figures 2 and 3 give the c.p.r.'s for S_1 and S_2 respectively for the condition of complete dominance. The no-dominance ($h = 0$) regressions for each c.p. group are also included. Table 10 gives algebraic values for c.p.r. coefficients, variances of F_1s, and other statistics for the three sets, S_1, S_2, and the condensed (ideal) set. It is obvious that the regression trends in all three sets reflect the dominance effect when "h" takes on some value other than zero. Although the same "h" value may be assigned to all three sets, the correspond-



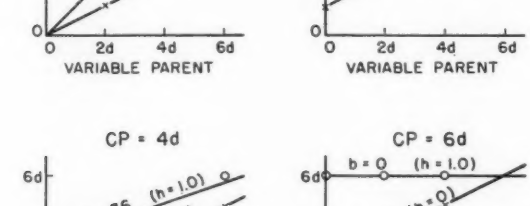


FIGURE 2.—Constant parent regressions for the four constant parent groups involved in set No. 1.

ing c.p.r. coefficients do not exactly agree. Thus, because of the sampling procedure operating in obtaining the set of parents, the c.p.r.'s do not exactly reflect the magnitude of dominance, although the inexactitude would probably be small if a wide range of character expression is exhibited among the parents. However, the fact that one has to deal with unpredictable points in the F_1 scatter, although causing some irregularities in the practical use of the c.p.r. technique, does offer another approach to the problem of dominance estimation.

This method of attack is to consider the deviations from regression and examine the components of F_1 variances. The general procedure due to FISHER (1918) of breaking down genotypic variances into additively genetic and non-additive portions is not of particular interest in this case. With

TABLE 10

Algebraic values for c.p.r. coefficients, variance among F_1s, and other statistics for the 4 c.p.groups in each of the 3 chosen sets, S_1, S_2, and

TABLE 10

Algebraic values for c.p.r. coefficients, variance among F_1s, and other statistics for the 4 c.p.groups in each of the 3 chosen sets, S_1, S_2, and condensed set.

	PARENTAL VALUE	c.p.r's.	VARIANCE AMONG F_1S	AMOUNT OF VARIANCE ATTRIBUTABLE TO REGRESSION $\frac{\{\sum xy\}^2}{\sum x^2}$	DEV'S FROM REG. $\sum d\bar{y} \cdot \bar{x}^2$	CORRELATION BETWEEN DOM. & BASIC GEN EFFECTS ρ
Set No. 1	$P_1=0$	$\frac{1+h}{2}$	$\frac{1}{3}(2d^2+4d^2h+2d^2h^2)$	$\frac{1}{3}(2d^2+4d^2h+2d^2h^2)$	0	+1
	$P_2=2d$	$\frac{1}{2}+\frac{h}{7}$	$\frac{1}{3}\left(\frac{14}{3}d^2+\frac{8}{3}d^2h+\frac{2}{3}d^2h^2\right)$	$\frac{1}{3}\left(\frac{14}{3}d^2+\frac{8}{3}d^2h+\frac{8}{21}d^2h^2\right)$	$\frac{6}{63}d^2h^2$	$+\sqrt{\frac{4}{7}}$
	$P_3=4d$	$\frac{1}{2}-\frac{h}{7}$	$\frac{1}{3}\left(\frac{14}{3}d^2-\frac{8}{3}d^2h+\frac{2}{3}d^2h^2\right)$	$\frac{1}{3}\left(\frac{14}{3}d^2-\frac{8}{3}d^2h+\frac{8}{21}d^2h^2\right)$	$\frac{6}{63}d^2h^2$	$-\sqrt{\frac{4}{7}}$
	$P_4=6d$	$\frac{1-h}{2}$	$\frac{1}{3}(2d^2-4d^2h+2d^2h^2)$	$\frac{1}{3}(2d^2-4d^2h+2d^2h^2)$	0	−1
		$b_2=-\frac{23h}{140d}$				
Set No. 2	$P_1=0$	$\frac{1+h}{2}$	$\frac{1}{3}(2d^2+4d^2h+2d^2h^2)$	$\frac{1}{3}(2d^2+4d^2h+2d^2h^2)$	0	+1
	$P_2=2d$	$\frac{1}{2}+\frac{3}{14}h$	$\frac{1}{3}\left(\frac{14}{3}d^2+4d^2h+2d^2h^2\right)$	$\frac{1}{3}\left(\frac{14}{3}d^2+4d^2h+\frac{6}{7}d^2h^2\right)$	$\frac{24}{63}d^2h^2$	$+\sqrt{\frac{3}{7}}$
	$P_3=4d$	$\frac{1}{2}-\frac{3}{14}h$	$\frac{1}{3}\left(\frac{14}{3}d^2-4d^2h+2d^2h^2\right)$	$\frac{1}{3}\left(\frac{14}{3}d^2-4d^2h+\frac{6}{7}d^2h^2\right)$	$\frac{24}{63}d^2h^2$	$-\sqrt{\frac{3}{7}}$
	$P_4=6d$	$\frac{1-h}{2}$	$\frac{1}{3}(2d^2-4d^2h+2d^2h^2)$	$\frac{1}{3}(2d^2-4d^2h+2d^2h^2)$	0	−1
		$b_2=-\frac{6h}{35d}$				
Condensed Set	$P_1=0$	$\frac{1+h}{2}$	$\frac{1}{3}(2d^2+4d^2h+2d^2h^2)$	$\frac{1}{3}(2d^2+4d^2h+2d^2h^2)$	0	+1
	$P_2=2d$	$\frac{1}{2}+\frac{1}{6}h$	$\frac{1}{3}\left(\frac{14}{3}d^2+\frac{28}{9}d^2h+\frac{14}{27}d^2h^2\right)$	$\frac{1}{3}\left(\frac{14}{3}d^2+\frac{28}{9}d^2h+\frac{14}{27}d^2h^2\right)$	0	+1
	$P_3=4d$	$\frac{1}{2}-\frac{1}{6}h$	$\frac{1}{3}\left(\frac{14}{3}d^2-\frac{28}{9}d^2h+\frac{14}{27}d^2h^2\right)$	$\frac{1}{3}\left(\frac{14}{3}d^2-\frac{28}{9}d^2h+\frac{14}{27}d^2h^2\right)$	0	−1
	$P_4=6d$	$\frac{1-h}{2}$	$\frac{1}{3}(2d^2-4d^2h+2d^2h^2)$	$\frac{1}{3}(2d^2-4d^2h+2d^2h^2)$	0	−1
		$b_2=-\frac{h}{6d}$				

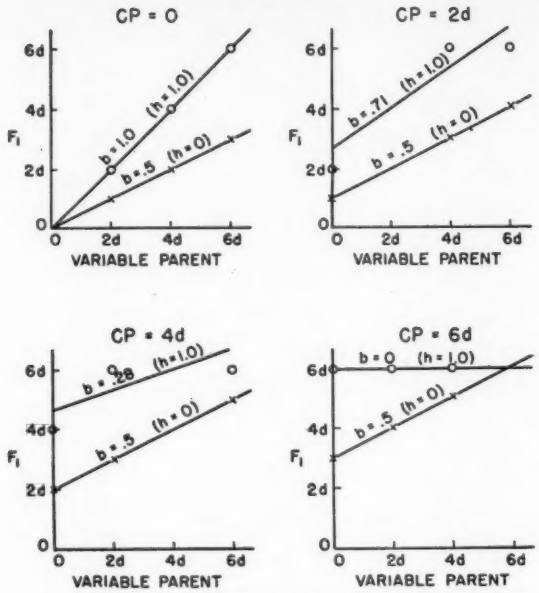


FIGURE 3.—Constant parent regressions for the four constant parent groups involved in set No. 2

homozygous parents and controlled matings a slightly different mathematical model may be used. The genotypic value of an F_1 is composed of two parts, a basic gene effect which is a function of "d," and a dominance effect which is a function of "dh." The variance among F_1s then involves a basic gene variance, a dominance variance, and a covariance, as these two effects may be correlated (see table 10). The partitioning of variances will be discussed after the next section.

2. Both dominance and epistasis (logarithmic case)

There are undoubtedly many ways that interaction between loci can be handled. The only general scheme that will be mentioned here will be that resulting from logarithmic gene action. When characters controlled by logarithmic or geometric gene action are measured in arithmetic values, it is obvious that gene interaction exists. In other words, the addition of a gene has an exponential or multiplicative effect, the value of which depends not only on its own activity but also on the presence of other genes in the genotype. By working out simple numerical examples, it may be readily observed that this general type of interaction as expressed on the arithmetic scale is detected by an increasing regression trend similar to that in the epistatic two-gene-pair model.

When, however, the data are transformed to logarithms, the gene action

itself is transformed to the additive scheme and the interaction disappears. This type of interaction is termed metrical bias and results when variations are measured on a different scale than is actually involved in the growth and expression of the character.

The problem then is first to choose whether the arithmetic or logarithmic scale will yield the best fitting model. This is taken up in the last section which discusses the general procedure for fitting gene models.

PARTITIONING OF VARIANCE OF F_1S WITHIN A CONSTANT PARENT GROUP

Let us assume that the proper transformation has been made so that between-loci effects as well as environmental effects are additive and independent. Consider, then, the i^{th} constant parent group, which has CP_i as the constant parent, VP_js where $j=1, 2, \cdots, n$ $(i \neq j)$ as variable parents, and F_{ij}s are F_1s resulting by crossing CP_i on all other variable parents.

The mathematical models involved are as follows:

$$CP_i = g_i + e_i$$

$$VP_j = g_j + e_j$$

$$F_{ij} = \frac{g_i}{2} + \frac{g_j}{2} + h_{ij} + e_{ij}$$

$$MP_{ij} = \frac{g_i}{2} + \frac{g_j}{2} + \frac{e_i}{2} + \frac{e_j}{2}$$

(where MP_{ij} is the midparent between CP_i and VP_j)

CP_i, VP_j, F_{ij} and MP_{ij} represent the phenotypic measurements for the particular trait considered on the i^{th} constant parent (CP_i), the j^{th} variable parent (VP_j), etc. g_i is the basic gene effect, which, in the gene model, is a function of "d" only. e_i, e_{ij} are individual errors. h_{ij} is the dominance effect for the F_{ij} and is a function of "dh" only.

Using the above mentioned models the F_{ij}s of the i^{th} constant parent group, associated variable parents and midparents may be represented as follows:

F_{ij}	VP_j	MP_{ij}
$F_{i1} = \frac{g_i}{2} + \frac{g_1}{2} + h_{i1} + e_{i1}$	$VP_1 = g_1 + e_1$	$MP_{i1} = \frac{g_i}{2} + \frac{g_1}{2} + \frac{e_i}{2} + \frac{e_1}{2}$
$F_{i2} = \frac{g_i}{2} + \frac{g_2}{2} + h_{i2} + e_{i2}$	$VP_2 = g_2 + e_2$	$MP_{i2} = \frac{g_i}{2} + \frac{g_2}{2} + \frac{e_i}{2} + \frac{e_2}{2}$
.	.	.
.	.	.
.	.	.
$F_{in} = \frac{g_i}{2} + \frac{g_n}{2} + h_{in} + e_{in}$	$VP_n = g_n + e_n$	$MP_{in} = \frac{g_i}{2} + \frac{g_n}{2} + \frac{e_i}{2} + \frac{e_n}{2}$

where $i \neq j$.

The following assumptions are used to obtain expected values of variances and covariances.

$E(g_k - \bar{g}_\cdot) = E(h_{ij} - \bar{h}_{i\cdot}) = E(e_m) = 0$ where $k = i$ or j; $m = i$, j or ij

$E(g_k - \bar{g}_\cdot)^2 = \sigma_g^2$; $E(h_{ij} - \bar{h}_{i\cdot})^2 = \sigma_h^2$; $E(e_m^2) = \sigma_e^2$

$E(g_k - \bar{g}_\cdot)(h_{ik} - \bar{h}_{i\cdot}) = \rho\sigma_g\sigma_h$, expected values of all other cross-products are zero.

$\bar{g}_\cdot$ is the mean genotypic value of the parents.

$\bar{h}_{i\cdot}$ is the mean dominance value of the F_1s in the i^{th} c.p. group.

The problem is to partition the variance of F_1s within each constant parent group and estimate the variances σ_g^2 and σ_h^2, and the correlation ρ. The variances and covariances are denoted as follows:

$V_{(F_{ij})}$ = variance of the F_1s within the i^{th} c.p. group.

$V_{(VP)}$ = variance of the variable parent values in the i^{th} c.p. group.

$V_{(MP)}$ = variance of midparental values of the variable parents and the constant parent.

$V_{(F_1-MP)}$ = variance of the differences between the F_1s and their corresponding midparents.

$CV_{(F_1 \times VP)}$ = covariance of the F_1s and their corresponding variable parents.

The expected values of these variances and covariances are as follows:

$$E\{V_{(F_{ij})}\} = (\tfrac{1}{4})\sigma_g^2 + \sigma_h^2 + \rho\sigma_g\sigma_h + \sigma_e^2 \qquad E\{V_{(F_1-MP)}\} = \sigma_h^2 + (\tfrac{5}{4})\sigma_e^2$$

$$E\{V_{(VP)}\} = \sigma_g^2 + \sigma_e^2$$

$$E\{V_{(MP)}\} = (\tfrac{1}{4})\sigma_g^2 + (\tfrac{1}{4})\sigma_e^2 \qquad E\{CV_{(F_1 \times VP)}\} = (\tfrac{1}{2})\sigma_g^2 + \rho\sigma_g\sigma_h.$$

Estimates of the genotypic components are obtained by equating observed and expected values.

$$(\tfrac{1}{4})\hat{\sigma}_g^2 = 1/4\{(V_{VP} - \hat{\sigma}_e^2)\} \qquad \widehat{\rho\sigma_g\sigma_h} = V_{(F_{ij})} - (\tfrac{1}{4})\hat{\sigma}_g^2 - \hat{\sigma}_h^2 - \hat{\sigma}_e^2$$

$$\hat{\sigma}_h^2 = V_{(F_1-MP)} - (\tfrac{5}{4})\hat{\sigma}_e^2.$$

The error component σ_e^2 is directly estimated from the analysis of variance of the replicated experiment and is the variance of parental and F_1 means (experimental error variance divided by the number of observations per parent or F_1).

The genotypic c.p.r. coefficient is:

$$b = \frac{(\tfrac{1}{2})\sigma_g^2 + \rho\sigma_g\sigma_h}{\sigma_g^2} = 1/2 + \rho\frac{\sigma_h}{\sigma_g} = 1/2 + \beta_{h \times g}$$

where $\beta_{h \times g}$ is the regression of the dominance contributions on the parental values.

These expected c.p.r. coefficients are estimated by:

$$\hat{b} = \frac{CV_{(F_1 \times VP)}}{V_{(VP)} - \hat{\sigma}_e^2}.$$

In general, we may assume that the best gene model is that which yields the most accurate prediction of F_1 values from those of its parents. This means that within a constant parent group the most desirable model or scale of measurement would be that which would minimize the mean square of deviations

from the constant parent regression. Thus, the separation of the F_1 variance into (1) the portion attributable to regression and (2) the portion due to deviations from regression, is of considerable importance as an aid in identifying the best fitting model. It is also of interest to point out the relationship existing between these portions and the components of the F_1 variance which have previously been discussed, namely σ_g^2, σ_h^2 and $\rho\sigma_g\sigma_h$. In terms of components the expected genotypic fraction of an F_1 variance attributable to the c.p.r. is as follows:

$$\frac{[(\tfrac{1}{2})\sigma_g^2 + \rho\sigma_g\sigma_h]^2}{\sigma_g^2} = (\tfrac{1}{4})\sigma_g^2 + \rho\sigma_g\sigma_h + \rho^2\sigma_h^2,$$

and the expected fraction due to deviations from regression is:

$$(1 - \rho^2)\sigma_h^2.$$

It is obvious that if $\rho=0$ then the basic gene variance and the mean square attributable to regression are equivalent; also the dominance component and deviations from regression are identical. If $\rho\neq 0$ this relationship does not hold.

In the gene model in which dominance exists but no between-loci-interaction, and all of the dominance effects are in the same direction of character expression, one can use variance components to estimate the value of "h." This necessitates theoretically the inclusion among the set of parents, of a completely recessive or completely dominant inbred or both. Then with their c.p. groups the dominance value to be associated with the gene model may be estimated as follows:

$$\hat{h} = \sqrt{\frac{\hat{\sigma}_h^2}{\hat{\sigma}_g^2}}.$$

Overdominance is indicated when $\hat{\sigma}_h^2$ is greater than $\hat{\sigma}_g^2$.

Tests of significance for the dominance component may be obtained by the following F test;

$$\hat{F} = \frac{V_{(F_1-MP)}}{(\frac{5}{4})\hat{\sigma}_e^2}$$

with $(n-1)$ degrees of freedom for the numerator where "n" is the number of F_1s in the c.p. group, and degrees of freedom for the denominator equal the degrees of freedom on which the experimental variance is based.

USE OF POTENCE RATIOS FOR CALCULATING APPROXIMATE MAGNITUDE OF DOMINANCE

In addition to the use of regression trends and variance component analyses, one can approximate a dominance value from the means of the non-segregating populations.

The proper procedure is to determine the appropriate scale which will transform between-loci gene action into the additive scheme and then compare

the F_1 means with mid-parental values to determine a "potence" value (h_p) as follows:

$$h_p = \frac{F_1 - M_p}{|P - M_p|}$$

where: M_p = midparent
P = parent (extreme)

This potence value will sufficiently approximate an average dominance value for the models discussed if one of the parents is at either extreme of character expression.

GENERAL PROCEDURE FOR FITTING GENE MODELS

The following steps are listed as the general procedure which may be used to fit the data to a specific gene model.

1. From arithmetic P_1 and F_1 means various statistics are calculated, such as (a) c.p.r. coefficients, (b) variance components, (c) deviations from the c.p.r., and (d) h_p values for the F_1s.
2. If the regression trend is decreasing, then positive dominance is assumed with arithmetic gene action. Amount of dominance is estimated by severity of regression trend, components, and h_p values.
3. If the regression trend is increasing, then the first problem arising is to distinguish between, (1) arithmetically cumulative action with negative dominance, and (2) logarithmically cumulative gene action with or without dominance.

This may be done by transforming the data to logarithms and comparing the various statistical values with the arithmetic analysis. Then,

(1) If on transformation to logarithms the c.p.r. trend is drastically reduced in comparison with the trend of the arithmetic c.p.r.s, this indicates that the arithmetic trend is due to logarithmic interaction (metrical bias) which can be removed by transformation.

(2) If on transformation to logarithms the deviations from regression are greatly reduced, this indicates that the deviations from regression are due mostly to metrical bias which disappears with transformation, and the best model would involve the logarithmic scale.

(3) If h_p values are irregular with arithmetic data and on transformation to logarithms become much more uniform, then this indicates that the better gene model is logarithmic.

(4) If in arithmetic analysis the c.p.r.s are all positive but increase sharply with largest valued c.p.r. considerably over $+1.0$, then this indicates logarithmic gene action. With negative dominance the c.p.r. coefficients should never exceed $+1.0$ (disregarding errors associated with c.p.r.'s) except in the case of overdominance, and usually one c.p.r. should have a negative value.

These four criteria may be used effectively to differentiate logarithmic from arithmetic gene action. When the proper basic gene action is decided upon, then estimates of amount of dominance are obtained by using the appropriate data (arithmetic or logarithmic) from the following statistics, (a) b_3, severity of regression trend, (b) components of variance, and (c) h_p values.

The development of the c.p.r. technique is obviously in its preliminary stages. However, when the trends of c.p.r. coefficients are used in combination with other approaches, a satisfactory analysis of the average gene action may be obtained. It should be emphasized again that as wide a range of character expression as possible is desirable. At least one extreme or both should be included among the parents so that regression trends, potence and component ratios are more reliable.

The real test of a new technique is in its practical application. HULL (1948) has reported some results in corn. Probably the most extensive use has been in the analysis of some ten characteristics in tomatoes (GRIFFING 1948). Here considerable success was obtained in fitting gene models to the data by the techniques outlined in this paper.

SUMMARY

An approach to the analysis of quantitative inheritance by use of the constant parent regression method is outlined. Variance components are also considered as an aid in understanding the relation between constant parent regressions and dominance deviations, and in evaluating the magnitude of dominance.

The approach is essentially that of constructing different gene models and then choosing the model that fits the data most accurately as determined by various statistics. As an introduction to the techniques, two-gene-pair models are considered first involving (1) additive gene action with dominance and no epistasis, and (2) gene-interaction involving both dominance and epistasis. The models are extended to "n" gene pairs with logarithmic gene action being the only type of gene interaction discussed. A procedure for differentiating the two basic models, arithmetic and logarithmic, is outlined together with an estimation of the amount of dominance on the closest fitting scale.

ACKNOWLEDGEMENTS

The author is indebted to DRS. JOHN W. GOWEN, SEWALL WRIGHT, and FRED H. HULL for constructive criticisms of the manuscript and to PROFESSOR OSCAR KEMPTHORNE for valuable aid in the statistical aspects of the paper.

LITERATURE CITED

BURDICK, A. B., 1949 Heterosis in the tomato; estimation of the relative contribution of epistasis and dominance. Unpublished Ph.D Dissertation, University of California.

FISHER, R. A., 1918 The correlation between relatives, on the supposition of Mendelian inheritance. Trans. roy. Soc. Edinb. **52**: 399–433.

GRIFFING, J. B., 1948 Statistical analysis of gene action in components of tomato yield. Unpublished Ph.D Dissertation, Ames, Iowa.State College, Ames, Iowa.

HULL, F. H., 1946 Maize Genetics Cooperation News Letter 20.

1947a Theoretical regression of F_n on homozygous parents with additive or complementary gene action. Genetics **32**: 90–91.

1947b Maize Genetics Cooperation News Letter 21.

1948 Maize Genetics Cooperation News Letter 22.

MATHER, K., 1949 Biometrical Genetics, pp. 82–97. Dover Publ. Inc., London.

RASMUSSON, J., 1933 A contribution to the theory of quantitative character inheritance. Hereditas **18**: 245–261.

QUANTITATIVE INHERITANCE OF FRUIT SIZE IN RED PEPPER (CAPSICUM FRUTESCENS L.)

IAN KHAMBANONDA[1]

Iowa State College, Ames, Iowa

Received November 14, 1949

THE objects of this experiment were to determine the pattern of inheritance of fruit-size characters, length, width, shape, and weight by successive selfings of a hybrid between two red pepper varieties which showed large differences: (1) to ascertain if shape genes are involved as such or if they are composites of length and width factors, (2) to observe the trend of means brought about by self-fertilization and to make deductions on actions of genes therefrom, (3) to estimate environmental, genotypic, and genetic variances in segregating generations, and (4) to estimate minimum number of genic differences required in the cross.

The usual genetic analysis of complex quantitative characters utilizes statistical methods by which genes and their combined effects are investigated as a whole. It involves fitting data to one model of gene action and disproving other alternatives. The disproof is cumbersome, is frequently disregarded, and so deductions are inconclusive.

In most instances in the past, materials were limited to parental, F_1, F_2, backcross generations, and selected advanced progenies. Often, results were inadequate for precise conclusion. The present study includes unselected F_3 and F_4 generations to supply the additional information needed in these analyses.

MATERIALS AND METHODS

Two commercial varieties of red pepper (*Capsicum frutescens* L.) were obtained from W. ATLEE BURPEE COMPANY of Philadelphia; Red Chili (P_1) has a small elongated fruit and Sunnybrook (P_2) a large oblate fruit. The varieties were naturally reproduced by self-fertilization. They were uniform in fruit and other morphological characteristics.

Plants were selfed once in 1940. Crosses were then made between the two varieties, using Red Chili as the female parent. F_1 plants were selfed for F_2. Seeds from sixty unselected F_2 plants were separately collected for sixty F_3 progenies. Four plants of each F_3 progeny were picked at random at the seedling stage and seeds were harvested and bulked by progeny for the F_4 field test. The production of seed was done in the greenhouse.

In the 1946 test, plants of all generations, P_1, P_2, F_1, F_2, F_3, and F_4 were started in the greenhouse and transplanted to the field. No selection was practiced.

The design of experiment was a randomized complete block. Four plots

[1] Present address: Department of Agriculture, Bangkok, Siam. Journal Paper No. J-1723 of the IOWA AGRICULTURAL EXPERIMENT STATION, Ames, Iowa. Project No. 250.

were randomized within a block; one plot with sixty F_2 plants; another plot with sixty F_3 plants, one plant per progeny; the third with sixty F_4 plants, one plant per progeny; and the fourth with a subplot of twenty P_1, another subplot with twenty P_2, and the third subplot with twenty F_1. Plants were randomized within plots and subplots; and subplots were randomized within a plot. F_3 and F_4 plants were labeled according to the progeny to which they belonged. The arrangement in a plot was ten plants per row spaced $1\frac{3}{4}$ feet within the row and $3\frac{1}{2}$ feet between rows, six rows per plot or two rows per subplot. Fifteen replications gave a total of 3,600 hills. Border rows were used.

Five fruits from each plant were harvested when ripe or turning red. Length was measured from the proximal to distal end of individual fruits with peduncle removed, and width at middle portion, to the nearest millimeter. The five measurements were averaged. The shape index was expressed as a ratio of length to width.

Dry and green weights of five fruits per plant were recorded to the nearest tenth of a gram and to the nearest gram respectively. Because, as shown below, a strong correlation is found between green weight and dry weight, and genetic analyses of the two lead to identical conclusions with regard to the number and properties of genes, only the green weight of fruit is reported here. The complete data may be obtained from IOWA STATE COLLEGE LIBRARY doctoral thesis 902. The total weight of five fruits per plant is presented in the results, and not the average fruit weight.

*Correlations between dry and green fruit weights within generations.**

P_1	P_2	F_1	F_2	F_3	F_4
.84	.48	.68	.89	.90	.90

* All highly significant beyond the 1 percent level.

GENETIC THEOREMS

A. *Means*

Ordinarily, the method of distinguishing multiplicative action from slight dominance of a gene is based on means.

Let P_1, P_2, and F_1 be the means of one homozygous parent, of the other homozygous parent, and of first generation hybrids respectively.

$$\text{Dominance on arithmetic scale} = \overline{F}_1 - \frac{\overline{P}_1 + \overline{P}_2}{2}$$

$$\text{Dominance on geometric scale} = \log \overline{F}_1 - \frac{\log \overline{P}_1 + \log \overline{P}_2}{2}$$

$$F_2 \text{ arithmetic mean} = \frac{\overline{P}_1 + \overline{P}_2 + 2\overline{F}_1}{4}$$

$$\log F_2 \text{ geometric mean} = \frac{\log \overline{P}_1 + \log \overline{P}_2 + 2 \log \overline{F}_1}{4}.$$

A conclusion may be drawn from the comparison of arithmetic and logarithmic estimates with the observed value of F_2. The calculations, however, are dependent on values of the homozygotes (P_1, P_2), the heterozygote (F_1), and the segregating population (F_2), all of which have different genetic backgrounds or substrates. These substrate factors may modify expressivity of genes. If they do so, conclusions derived from P_1, P_2, F_1, and F_2 data may be misleading, whereas F_2, F_3, and F_4 results should be preferred since individuals of the latter generations have comparatively similar samples of the genetic substrates.

As criteria for distinguishing effects of genes, the variabilities and the skewness of distributions of P_1, P_2, and F_1 populations may also be used.

Variations within P_1, P_2, and F_1 are due to environmental influence on the character. If the variations in all three populations are approximately equal, the effects of environment are additive. If they become equal by transforming data to a logarithmic scale, then the effects of environment, and probably but not necessarily those of genes, are multiplicative. As effects of genes and environment are often not in the same direction, the test is not conclusive.

The skewness of distributions within P_1, P_2, and F_1 indicates whether environmental variations are additive or multiplicative. However, the third moment statistic, especially of distributions of segregating generations, may be exaggerated by the infrequent inclusion of extreme individuals so that it does not always measure skewness as such.

An effective method of differentiating genic manifestations is the examination of the trend of means of successively selfed generations after hybridization.

Ignoring various genetic substrates and assuming no epistasis, suppose a character is affected by two pairs of genes:

$$AA = 2x_1 \qquad Aa = x_1 + d_1 \qquad aa = 0$$

$$BB = 2x_2 \qquad Bb = x_2 + d_2 \qquad bb = 0.$$

Symbols x_1, x_2, d_1, and d_2 may represent any real values. If d_1 and d_2 are positive, dominance for the large value is indicated; if d_1 and d_2 are negative, dominance is for the small value. If d_1 is positive and d_2 negative, both dominances are present; if d_1 and d_2 are zero, dominance is absent.

Let $P_1 = AAbb$ and $P_2 = aaBB$
Mean $P_1 = 2x_1$ and mean $P_2 = 2x_2$
$1/2(\text{mean } P_1 + \text{mean } P_2) = x_1 + x_2$
Mean $F_1 = x_1 + x_2 + d_1 + d_2$
Mean $F_2 = x_1 + x_2 + 1/2(d_1 + d_2)$
Mean $F_3 = x_1 + x_2 + 1/4(d_1 + d_2)$
Mean $F_4 = x_1 + x_2 + 1/8(d_1 + d_2)$
Mean $F_\infty = x_1 + x_2$

The formulae may be extended to r factors and n generations of self-fertilization:

$$\text{Mean } F_n = \sum_{i=1}^{r} x_i + \frac{1}{2^{n-1}}\left(\sum_{i=1}^{r} d_i\right).$$

It may be proved that when there is no selection, linkage does not change the means of generations. Although it alters genotypic ratios and variances, the means remain the same as if linkage is absent.

By continued selfing without selection, the mean of any one generation approaches the mean of parents by one-half of the difference between the mean of the preceding generation and the mean of parents.

$$\bar{F}_{n-1} - \bar{F}_n = 1/2\left(\bar{F}_{n-1} - \frac{\bar{P}_1 + \bar{P}_2}{2}\right),$$

where n = generations of selfing.

$$\bar{F}_2 - \bar{F}_3 = 1/2\left(\bar{F}_2 - \frac{\bar{P}_1 + \bar{P}_2}{2}\right)$$

$$\bar{F}_{n-1} - \bar{F}_n = 1/2(\bar{F}_{n-2} - \bar{F}_{n-1})$$

$$\bar{F}_3 - \bar{F}_4 = 1/2(\bar{F}_2 - \bar{F}_3).$$

In the above formulae, if $\sum d_i$ is positive (excess of dominance of *large* values), even if some d_i are negative, the trend of generation means is *decreasing*.

A common problem in analysis is the interpretation of positive skewness of F_2 distributions, which may be explained by either multiplicative effects of genes or dominance of small values. If dominance is predominantly for *small* values ($\sum d_i$ negative), the trend of means of successively selfed generations is invariably *increasing*. If it is not increasing, dominance of small values cannot be the cause of skewness. An alternative explanation, the multiplicative effects of genes can be considered; and the original data should be transformed to logarithms for analysis.

The formulae hold true for all degrees of dominance, partial, complete, or overdominance, positive or negative direction, or combinations of them. The sum of dominant deviations, $\sum d_i$, may be estimated from means of all generations.

As a corollary, if $\sum d_i$ is zero, in which dominance of small and large is balanced, or dominance is absent, the average of parental means equals the mean of F_1, F_2, F_3, $\cdots$, F_n. Selfing without selection does not increase or decrease the mean of any generation.

Epistasis or interaction of non-allelic genes would shift the means in one direction or the other essentially in the same manner as dominance, which is interaction of allelic genes. In inhibiting interaction, which is comparable to dominance of small values, the means of successively selfed generations would tend to increase; whereas in complementary action, which is comparable to dominance of large values, they would decrease. The rate of shift depends on the kind and magnitude of epistasis in question; and no generalization can be formulated.

B. *Variances*

Variances of P_1, P_2, and F_1, which are entirely environmental, may be equal. POWERS (1942) finds that, in the tomato, they are proportional to generation means. CHARLES and SMITH (1939) believe that standard deviations are correlated with means. GUSTAFSSON (1946) finds F_1 more variable than P_1 and P_2, whereas it is a common experience among maize breeders that in some characters as date of tasseling, inbreds are more affected by climatic changes than F_1. In cotton, PANSE (1940) does not find any relationship between variances and means of staple length.

If the environmental variance of segregating generations can be estimated from P_1, P_2, and F_1, then genotypic variance (H) of F_2, F_3, and F_4 is the difference between total variance (T) and environmental variance (E), assuming the interaction or covariance (HE) is zero. Genotypic variance (H) may be broken down to additively genetic variance (G) and variances ascribed to dominance (D), epistasis (I), and linkage (L).

$$T = H + E = G + D + I + L + E.$$

When homozygous parents are used, the genotypic variance of non-segregating generations is zero.

$$H(P_1) = H(P_2) = H(F_1) = 0.$$

With selfing, the genotypic variances of segregating generations may be formulated as follows:

If dominant homozygote *AA* is 2x, heterozygote *Aa* is x+d, and recessive homozygote *aa* is 0,

Variance of F_2 is $1/4(2x^2+d^2)$

Variance of F_3 is $3/16(4x^2+d^2)$

Variance of F_4 is $7/64(8x^2+d^2)$

Variance of F_n is

$$[2^{n-1} - 1]\left[\frac{x^2}{2^{n-1}} + \frac{d^2}{4^{n-1}}\right],$$

where n = generations of selfing.

With r independent genes with no epistatic effects,

$$H(F_n) = [2^{n-1} - 1]\sum_{i=1}^{r}\left[\frac{x_i^2}{2^{n-1}} + \frac{d_i^2}{4^{n-1}}\right].$$

(1) With no epistasis or linkage, the genotypic variances of whole segregating generations may be compared.

(a) If dominance is absent, d = 0, the heterozygote being mid-way between parental homozygotes.

$$\frac{G(F_n) - G(F_{n-1})}{G(F_{n-1}) - G(F_{n-2})} = 1/2.$$

where n = generations of selfing.

$$G(F_3) = 1.50\ G(F_2)$$

$$G(F_4) = 1.75\ G(F_2).$$

(b) If all factors are completely dominant, for large ($d=x$) or for small ($d=-x$), or for both large and small values.

$$\frac{H(F_n) - H(F_{n-1})}{H(F_{n-1}) - H(F_{n-2})} = 1/4$$

$$H(F_3) = 1.25\ H(F_2)$$

$$H(F_4) = 1.3125\ H(F_2).$$

(c) If some factors are completely dominant while others are not, or if factors are partially dominant ($0<d<|x|$), or combinations thereof,

$$1/4 < \frac{H(F_n) - H(F_{n-1})}{H(F_{n-1}) - H(F_{n-2})} < 1/2.$$

(d) As selfing continues, overdominance ($d>|x|$) of some size, such as $d>|2x|$, decreases genotypic variance,

$$H(F_n) < H(F_{n-1}).$$

The decrease may be roughly visualized by the fact that selfing lowers the proportion of heterozygotes, which, being overdominant, have extreme values away from the generation mean.

(2) With the same notation for a segregating gene pair,

Variance of F_2 is $1/4(2x^2+d^2)$

Mean variance within F_3 progenies is $1/8(2x^2+d^2)$

Mean variance within F_4 progenies is $1/16(2x^2+d^2)$

For any number of independently assortive gene pairs with no epistasis, mean genotypic variance within F_3 progenies equals one-half of F_2 genotypic variance, and mean genotypic variance within F_4 progenies is one-half of that within F_3 progenies. It may be generalized that mean genotypic or genetic variance within progenies of any one generation of selfing beyond F_3 is one-half of that within progenies of the preceding generation.

(3) With the same symbols,

Variance of F_2 is $1/4(2x^2+d^2)$

Variance of means of F_3 progenies is $1/4(2x^2+1/4d^2)$

Variance of means of F_4 progenies is $1/4(2x^2+1/16d^2)$

Variance due to dominance (D) may be estimated as:

Variance of F_2 − Variance of means of F_3 progenies $= 3/16d^2$

Variance of means of F_3 progenies − Variance of means of F_4 progenies $= 3/64d^2$

If r pairs of genes are not linked with no epistatic deviations, the above two equations become $3/16\sum_{i=1}^{r} d_i^2$ and $3/64\sum_{i=1}^{r} d_i^2$ respectively. If dominance of each gene is also equal in magnitude, d, the values finally become $3/16rd^2$ and $3/64rd^2$.

If dominance is absent, the genetic variance of F_2, $G(F_2)$, is equal to genetic variance of means of F_3, or F_4, progenies.

(4) The regression of F_3 progenies on F_2 parents has been used by PANSE (1940) to measure heritability or genetic variance (G). But the estimation of genetic variance of F_3 from genetic variance of F_2 grown in the previous year is not entirely correct unless genotypic manifestations of F_2 and F_3 are similarly affected by environment from year to year. In a majority of agronomic characters, it has been found that interaction between genotypes and year, or covariance (HE), is often significantly large. The interaction may be eliminated by use of F_3 and F_4 planted at the same time as is done in this experiment.

For a single gene pair, if AA is 2x, Aa is x+d, and aa is 0,

Regression of F_3 progeny means on F_2 parents

$$= \frac{1/4(2x^2 + 1/2d^2)}{(\text{Genotypic variance of } F_2 + \text{Environmental Variance})}$$

$$= \frac{1/4(2x^2 + 1/2d^2)}{\text{Total variance of } F_2}.$$

Regression of F_4 progeny means on F_3 progeny means

$$= \frac{1/4(2x^2 + 1/8d^2)}{\begin{array}{l}(\text{Genotypic variance of } F_3 \text{ progeny means} \\ \quad + \text{Environmental variance of } F_3 \text{ progeny means})\end{array}}$$

$$= \frac{1/4(2x^2 + 1/8d^2)}{\text{Total variance of } F_3 \text{ progeny means}}.$$

If dominance is absent, both values of the regression would measure the fraction of genetic variance, $1/2x^2$. With dominance, the latter, the regression of F_4 on F_3, is a closer estimate of genetic portion of variance than the former. Assuming no epistasis for r pairs of genes, if dominance is incomplete, in which $1/8d_i^2$ is negligibly small when compared with $2x_i^2$, the regression of F_4 on F_3 may be used to estimate heritability as is done in this experiment.

(5) Qualitative factors have been demonstrated to exhibit several kinds of epistasis. Unless epistatic manifestations are outstanding, a gross study like the present one cannot identify them. In quantitative characters, dominance and epistasis give similar statistical results. An effect ascribed to epistasis such as heterosis may well be explained by dominance, and *vice versa*.

(6) Depending on phase, linkage has a role in variance of segregating populations. In coupling phase, it increases the variance; in repulsion phase, the reverse is true. The magnitude depends on recombination values and generations of inbreeding.

With continued selfings, the change in variance attributed to linkage is comparatively large in the first few generations, especially when recombination values are high. After three or four self-fertilizations, which have rapidly brought homozygosis, the variance becomes constant as equilibrium is being reached when the proportion of gametes in coupling and repulsion phases is

constant. To confirm the statement, formulae by LINDSTROM (1946) may be cited:

$$r_n = 2(r_0 + s_0)r_{n-1} - (r_0 - s_0)^{n-1}$$
$$s_n = 2(r_0 + s_0)s_{n-1} + (r_0 - s_0)^{n-1}$$

where r:s is the gametic ratio, s=1, and n=generation of self-fertilization.

$$r_\infty = \frac{r_0 + 1}{r_0 + 3} \text{ at equilibrium.}$$

If there is linkage, the mean variance within F_3 progenies is not expected to be one-half of F_2 variance, (c.f. PANSE 1940). Assuming *Aa* with variance w, *Bb* with variance z, and the two genes linked with no epistatic interaction, the variance contributed by a double heterozygote *AaBb* is not (w+z) but (w+z+covariance wz). The covariance component is ascribed to linkage; it is positive in case of coupling and negative in case of repulsion. Hence, the relation, that the mean variance within F_3 progenies is one-half of F_2 variance, or that the mean variance within F_4 progenies is one-half of that within F_3 progenies, is true only when linkage or epistasis is absent. The variance of F_3 or F_4 variances is also dependent upon intensity and phase of linkage.

C. *Number of Genes*

The conventional estimation of number of genic differences in any cross is based on a ratio obtained by arbitrary apportionment of F_2 when separation of phenotypes is possible in a multimodal distribution. However, such simple cases are rare. Even then, the conclusion depends largely on how F_2 individuals are classified into groups.

For unimodal distributions, several investigators have presented methods of analysis which are essentially based on the expansion of a binomial $(x+y)^{2r}$, where x and y are allelic gene values and r is the number of gene pairs. The genes are assumed to be independently assortive, having equal additive effects with no dominance or epistasis.

According to CASTLE (1921) and WRIGHT (1934), if r is the number of pairs of not linked genes with additive action, no dominance or epistasis, and x_i is a single gene effect, then the genetic range (Δ) is $2\sum_{i=1}^{r} x_i$, and the F_2 genetic variance, $G(F_2)$, is $1/2\sum_{i=1}^{r} x_i^2$.

$$\frac{(\text{Genetic range})^2}{\text{Genetic variance of } F_2} = \frac{\Delta^2}{G(F_2)} = \frac{4\left(\sum_{i=1}^{r} x_i\right)^2}{1/2\sum_{i=1}^{r} x_i^2}.$$

If the genes have equal effects, x, in which a minimum number of genes is estimated, then

$$\frac{\Delta^2}{G(F_2)} = \frac{4r^2x^2}{1/2rx^2} = 8r, \quad \text{also} \quad \frac{\Delta^2}{G(F_3)} = \frac{16r}{3}, \quad \text{and} \quad \frac{\Delta^2}{G(F_4)} = \frac{32r}{7}.$$

The difference between homozygous parental values has often been used as the genetic range. SHULL (1921) points out that this is incorrect for the parents need not be extreme genotypes of the range, and in quantitative characters, the probability of their being so is very small. If the number of genes is large, samples of F_2 also are unlikely to include the extremes.

With no linkage or epistasis, if all dominance is complete in only one direction and the genes are additive, the genetic range is

$$2\left(\bar{F}_1 - \frac{\bar{P}_1 + \bar{P}_2}{2}\right).$$

If the dominant phenotype of a gene pair is $2x_i$, recessive 0, and the number of gene pairs r, then the genetic range (Δ) is $2\sum_{i=1}^{r} x_i$, and the F_2 genotypic variance, $H(F_2)$, is $3/4\sum_{i=1}^{r} x_i^2$.

$$\frac{(\text{Genetic range})^2}{\text{Genotypic variance of } F_2} = \frac{\Delta^2}{H(F_2)} = \frac{4\left(\bar{F}_1 - \frac{\bar{P}_1 + \bar{P}_2}{2}\right)^2}{H(F_2)} = \frac{4\left(\sum_{i=1}^{r} x_i\right)^2}{3/4\sum_{i=1}^{r} x_i^2}.$$

With equal gene effects, x,

$$\frac{\Delta^2}{H(F_2)} = \frac{16r}{3}, \qquad \frac{\Delta^2}{H(F_3)} = \frac{64r}{15}, \quad \text{and} \quad \frac{\Delta^2}{H(F_4)} = \frac{256r}{63}.$$

If dominant deviations are both positive and negative in directions, the genetic range will be larger than

$$2\left(\bar{F}_1 - \frac{\bar{P}_1 + \bar{P}_2}{2}\right).$$

Selection for extreme values for several generations will give a close estimate of the range.

With another approach, PANSE (1940) estimates genetic variance of F_2 by regression of F_3 progenies on F_2, and calculates the "effective" number of factors as a ratio of squared genotypic variance within F_3 progenies to variance of the genotypic F_3 variances. As he cannot find genotypic variance, he substitutes genetic variance for genotypic. He proceeds to fit the results to various models of genes, some with dominance and multiplicative effects, which would contradict the definition of genetic variance, which he has actually calculated.

Assuming no linkage or epistasis, and all r factors segregating with equal variance w in F_2, PANSE proves that

$$\text{Mean genotypic variance within } F_3 \text{ progenies} = \bar{H}(F_3) = \frac{rw}{2}$$

$$\text{Variance of genotypic variances within } F_3 \text{ progenies} = V[\bar{H}(F_3)] = \frac{rw^2}{4}$$

$$\frac{[\bar{H}(F_3)]^2}{V[\bar{H}(F_3)]} = \frac{4r^2w^2}{4rw^2} = r = \text{"effective" number of genes.}$$

For F_4 progenies, it may be proved that:

$$\text{Mean genotypic variance within } F_4 \text{ progenies} = \bar{H}(F_4) = \frac{rw}{4}$$

$$\text{Variance of genotypic variances within } F_4 \text{ progenies} = V[\bar{H}(F_4)] = \frac{3rw^2}{16}$$

$$\frac{[\bar{H}(F_4)]^2}{V[\bar{H}(F_4)]} = \frac{r^2w^2}{3rw^2} = \frac{r}{3}.$$

RESULTS AND DISCUSSION

A. *Fruit Length and Width*

Frequency distributions of fruit length and width in all generations are presented in figures 1 and 2, the means in table 1, and correlations in table 2.

TABLE 1

Fruit length and width.

GENERATION	df	MEAN LENGTH	MEAN WIDTH
		mm	mm
P_1 (Red Chili)	285	43.5	8.4
P_2 (Sunnybrook)	283	48.2	65.6
F_1	285	51.3	25.1
F_2	885	48.9	25.0
F_3	883	51.5	23.4
F_4	882	50.1	23.2

For length, the F_1 mean is larger than that of either parent. The F_2, F_3, and F_4 distributions are positively skewed, and are transgressive far beyond parental and F_1 values. Fruit width of F_1 is intermediate between P_1 and P_2. The F_2, F_3, and F_4 individuals are distributed between parental values, but the distributions cannot be sharply divided into classes. The small parent P_1 is recovered in the selfed generations, whereas the large width of P_2 is not.

In table 2, the correlation between length and width is positive in P_1, P_2, and F_1, but is negative in F_2, F_3, and F_4 generations. This is one of the reasons offered by SINNOTT (1935) for a theory that factors controlling shape exist. In non-segregating populations, in which variation is not genetic, an environmental deviation in one dimension, length, may be positively correlated with that in the other dimension, width. In segregating generations, if length and width genes are not linked, the genetic correlation should be zero. If they are genetically linked, the correlation, its magnitude depending on crossover

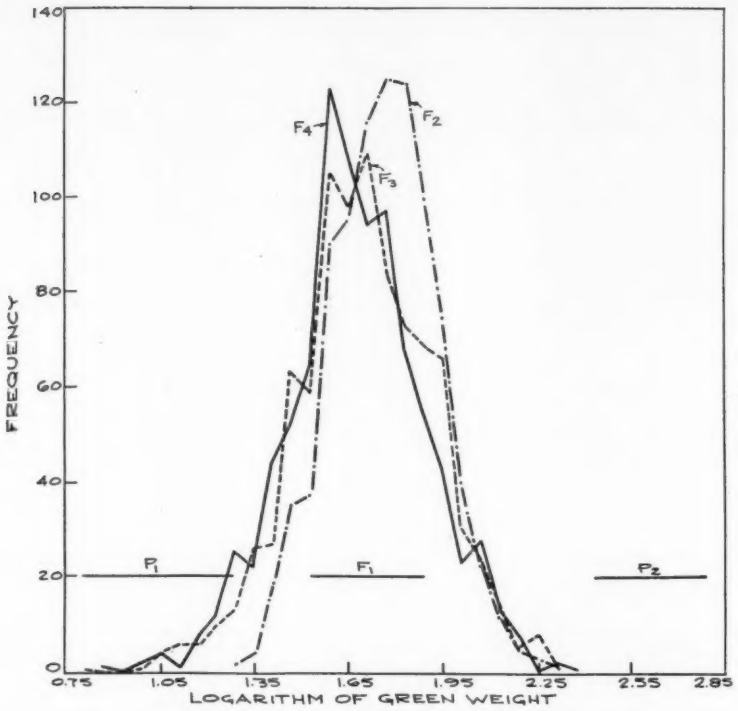


FIGURE 1.—Frequency distributions of fruit lengths within generations.

TABLE 2

Correlations within generations.

CORRELATION*	P_1	P_2	F_1	F_2	F_3	F_4
Length and Width	.56	.15	.12	−.37	−.24	−.26
Length and Weight	.73	.53	.39	.12	.17	.17
Length and Weight Independent of Width	.57	.64	.39	.68	.73	.69
Width and Weight	.68	.75	.53	.77	.85	.82
Width and Weight Independent of Length	.47	.80	.52	.88	.93	.91
Shape and Weight	−.10	−.03	−.21	−.26	−.29	−.26

* Significance for P_1, P_2, and F_1 .15 at 1 percent level
for F_2, F_3, and F_4 .08 at 1 percent level

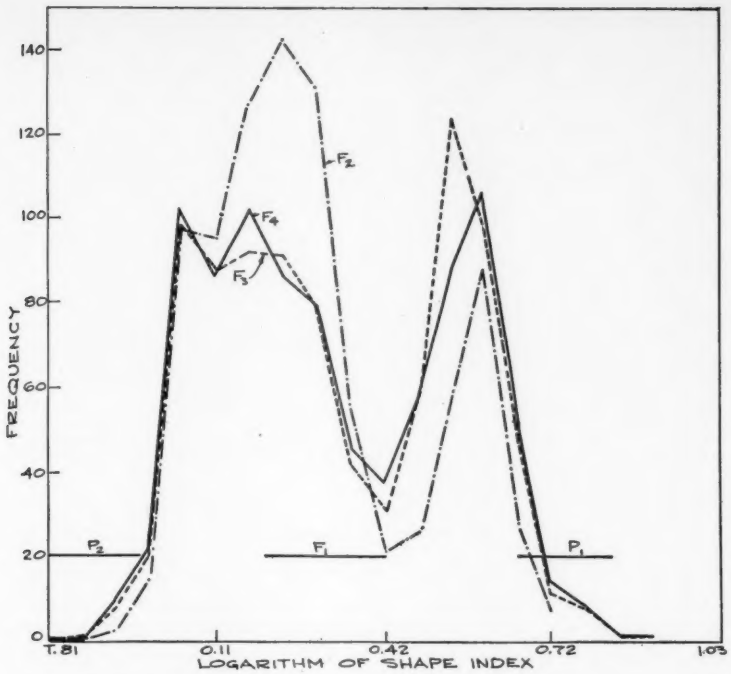


FIGURE 2.—Frequency distributions of fruit widths within generations.

values, should be higher in F_2 than in F_3, and higher in F_3 than in F_4. In case of complete linkage, in which length and width together express shape, a strong correlation is expected. The observed correlations between length and width, $-.37$, $-.24$, and $-.26$, though highly significant, are of only mediocre magnitude.

B. *Fruit Shape*

Fruit shape, as expressed by the ratio of length to width, is negatively correlated with fruit weight (table 2). Although the correlations in the segregating generations are highly significant, the values ($-.26$, $-.29$, and $-.26$) are relatively small. If shape factors are linked with weight factors, most of them must be but loosely so.

Sharp segregations of shape indices in the F_2, F_3, and F_4 (figure 3) give evidence that shape genes are major operative factors, which regulate the relative growths of length and width. The final fruit length and width are largely manifestations of interaction between fruit weight and shape. It seems justified to analyze length and width as a ratio, or shape index, rather than to investigate them separately. Shape factors in plants, indeed have been identified by several investigators. LINDSTROM (1927) located a shape gene linked with

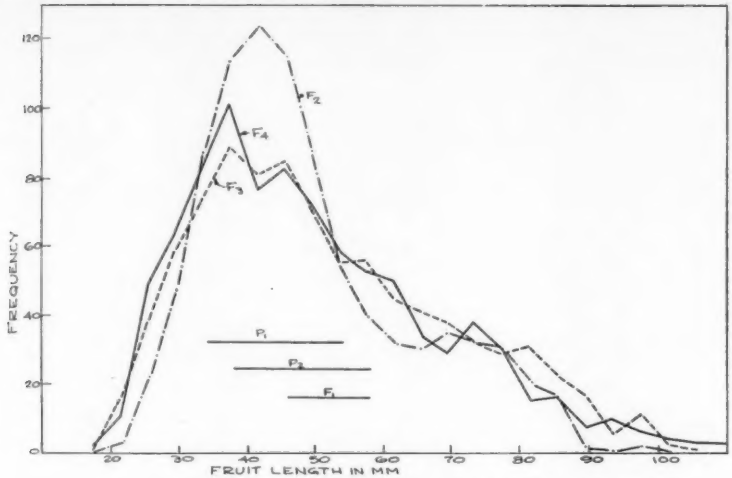


FIGURE 3.—Frequency distributions of logarithms of fruit-shape indices within generations

"dwarf" and "peach" on first chromosome of the tomato. In the red pepper, the effects of genes determining fruit shape were demonstrated by KAISER (1935). He observed that the ovaries from primordium stage to anthesis were essentially similar in shape. After anthesis, he found two rates of relative growth in length and width, and could divide them into a simple Mendelian ratio of three to one in the F_2. In other species, tomato (YEAGER 1937), squash (SINNOTT and DURHAM 1929), cucumber (HUTCHINS 1934), and watermelon (WEETMAN 1937), fruit-shape dissimilarities were shown to be fixed before anthesis, in the ovary primordia.

It is desirable first to decide whether the analysis should be made on the arithmetic or logarithmic scale. From table 3, the trend of means of F_1 to F_4 generally increases. The F_1 (2.04) is lower than the arithmetic average of P_1 and P_2 (2.97). These facts, however, do not distinguish dominance of small shape index from multiplicative action of genes. The logarithmic means also do not serve as a criterion.

Variance of P_1, which has high shape index (elongated fruit), is the largest of all variances of non-segregating populations, that of F_1, intermediate, and that of P_2 (oblate), the smallest. The ratios of variances to corresponding means follow an exponential curve. A regression line may be fitted to standard deviations and means for estimating environmental variation of F_2, F_3, and F_4, but, by transforming data to logarithms, the variances of P_1, P_2, and F_1 are nearly equal, averaging .0011. Thus, environmental variation is multiplicative, and probably so are gene effects. Relying on this parallelism, the author decided to use logarithms; hence, multiplicative gene actions are assumed.

Logarithmic distributions of F_2, F_3, and F_4 are positively skewed, each with three modes, suggesting a major gene for fruit shape with incomplete domi-

nance of oblate type. The homozygous dominant class cannot be isolated from the heterozygotes, but the recessives can be separated from the other two types. When the separation is made, the observed frequencies may be tested against the monohybrid ratios.

	Frequencies of Shape Index Log Class Intervals		*Expected Ratio*	x^2
	−.22 to .40	.41 to .88		
F_2	679	221	3:1	0.10
F_3	538	360	5:3	2.51
F_4	534	363	9:7	3.81

The last value of chi-square (3.81) is close to 3.84, the significant level at 5 percent probability. This test of significance should not be too strongly emphasized for deviations from expected ratios depend much upon the partitioning of numbers in the class between the two groups. However, the segregation characteristic of a single gene pair is fairly sharp. If modes can be used to evaluate genotypes, which is a reasonable supposition in this case, then the variances on a logarithmic scale may be calculated for this monogenic segregation.

Oblate (*OO*) = .06 (shape index 1.15)
Heterozygote (*Oo*) = .18 (shape index 1.51)
Elongated (*oo*) = .54 (shape index 3.43)
Genotypic variance of F_2 = .0324
Genotypic variance of F_3 = .0459
Genotypic variance of F_4 = .0520

The calculated genotypic variances of F_2 and F_3 are slightly lower than the observed (.0334 and .0460), whereas the calculated genotypic variance of F_4 is higher than the observed (.0466), leaving no variance for any other genes. However, the dominants (*OO*) and the recessives (*oo*) do not exactly correspond to the P_2 mean (−.133) and the P_1 (.714) respectively. It may be inferred that the difference between the genotype *OO* (at modal value of .06) and P_2 type, or that between the genotype *oo* (at modal value of .54) and P_1 type, is due to environmental variation and to the expression of this gene in dissimilar genetic substrates.

The heterozygous value (*Oo*) minus the average of homozygous values (*oo* and *OO*) gives the dominant deviation, d.

$$d = .18 - \frac{.54 + .06}{2} = -.12$$

$$d^2 = .0144.$$

With the observed generation means, the calculated dominant deviation is far from expected:

$$d = 4(F_2 \text{ mean} - F_3 \text{ mean}) = -.200$$

$$d = 8(F_3 \text{ mean} - F_4 \text{ mean}) = .040$$

By subtracting the averaged environmental variance (.0011) from the total variances of F_2, F_3, and F_4, the genotypic variances may be computed as listed in table 3. The ratios of genotypic variances are as follows:

$$\frac{H(F_3) - H(F_2)}{H(F_2) - H(F_1)} = \frac{.0460 - .0334}{.0334} = .38$$

$$\frac{H(F_4) - H(F_3)}{H(F_3) - H(F_2)} = \frac{.0466 - .0460}{.0460 - .0334} = .05.$$

The value of .38 which is between one-half and one-quarter is expected when dominance is incomplete.

Variance of F_2 − Variance of F_3 progeny means

$$= 3/16d^2 = .0334 - .0236$$

$$d^2 = .0523.$$

Variance of F_3 progeny means − Variance of F_4 progeny means

$$= 3/64d^2 = .0236 - .0198$$

$$d^2 = .0810.$$

The two values of d^2 are higher than the theoretical value of .0144 estimated from genotypes.

From table 3, the regression of F_4 on F_3 progeny means is .68. The genetic variance between means of F_3 progenies, therefore, is .68(.3775/15) or .0171, which is also the genetic variance of F_2. Using WRIGHT's formula, if parental means are taken as the genetic range (.847), and if genes are not linked with equal effects, then,

$$\text{Minimum number of genes} = \frac{(\text{Genetic range})^2}{8\ (\text{Genetic variance of } F_2)}$$

$$= \frac{(.847)^2}{8(.0171)} = \text{about } 5.$$

Using the difference between homozygous genotypes as genetic range (.48),

$$\text{Minimum number of genes} = \frac{(.48)^2}{8(.0171)} = 1.7.$$

Following PANSE's approach, if there is no epistasis or linkage, the mean genotypic variance within F_3 progenies is 1/2(.0334) or .0167. Variance of this variance is .000302. The "effective" number of genes is .0167/.000302 or approximately 55, which is quite high. With the use of F_4 values, the number is $1/4(.0334) \times 3/.000297$ or 84, where .000297 is variance of F_4 variance.

The variance of variance may be inaccurate as the plants within each progeny, with the present experimental design, are confounded with block variation. Also, it may be added that an underestimate of environmental variation will give too high an answer for the number of genes.

TABLE 3

Mean and variances of fruit-shape indices.

	df	ARITHMETIC ANALYSIS		LOGARITHMIC ANALYSIS			
		MEAN	MEAN SQUARE	MEAN	MEAN SQUARE	GENOTYPIC VARIANCE	GENETIC VARIANCES
		mm		log			
Blocks	14		0.7586		.0230		
Error	42		0.6800		.0222		
P_1 (Red Chili)	285	5.19	0.1428	.714	.0011		
P_2 (Sunnybrook)	283	0.74	0.0031	−.133	.0011		
F_1	285	2.04	0.0225	.309	.0011		
F_2	885	2.10	0.9943	.280	.0345	.0334	.0171
F_3 total	883	2.42	1.4299	.330	.0471	.0460	1.50 (.0171)
between progenies	59		11.2070		.3775		
within progenies	824		0.7298		.0234	.50 (.0334)	.50 (.0171)
F_4 total	882	2.40	1.4741	.325	.0477	.0466	1.72 (.0171)
between progenies	59		9.0846		.3250		
within progenies	823		0.9285		.0279	.25 (.0334)	.25 (.0171)

Logarithmic Analysis:

Average environmental variance = .0011.

Genotypic variance = total variance − environmental variance of .0011.

Regression of F_4 progeny means on F_3 progeny means = .68.

Genetic variance between F_3 progeny means $= .68\left(\frac{.3775}{15}\right) = .0171.$

Genotypic variance between F_3 progeny means = 1/15(.3775 − .0234) = .0236.

Genotypic variance between F_4 progeny means = 1/15(.3250 − .0279) = .0198.

C. *Fruit Weight*

Fruit weight measures the weight of five fresh ripe fruits per plant. Distributions of fruit weights in various generations are presented in figure 4, and the statistics in table 4. Since block differences are insignificant, the frequencies can be combined in plotting the curves.

With arithmetic analysis, the distributions are unimodal, and those of F_2, F_3, F_4 positively skewed, indicating dominance of small size or multiplicative variations. The small parent P_1 is recovered in the segregating generations, but the large P_2 is not. The F_1 is closer to P_1 than to P_2 and it is much smaller than the average of the two parents (222.0).

Variances of P_1, P_2, and F_1 increase exponentially with means, thus the environmental variation is multiplicative. The decreasing trend of means from F_2 to F_4 is characteristic of gene actions with excess of dominance of large or epistasis of large size. The hypothesis that dominance of small fruit weight is the cause of skewness would be untenable. The alternative hypothesis, the multiplicative gene actions, may be accepted. However, it is noted that the F_1 mean is smaller than the F_2 mean, which is contrary to the expectation when

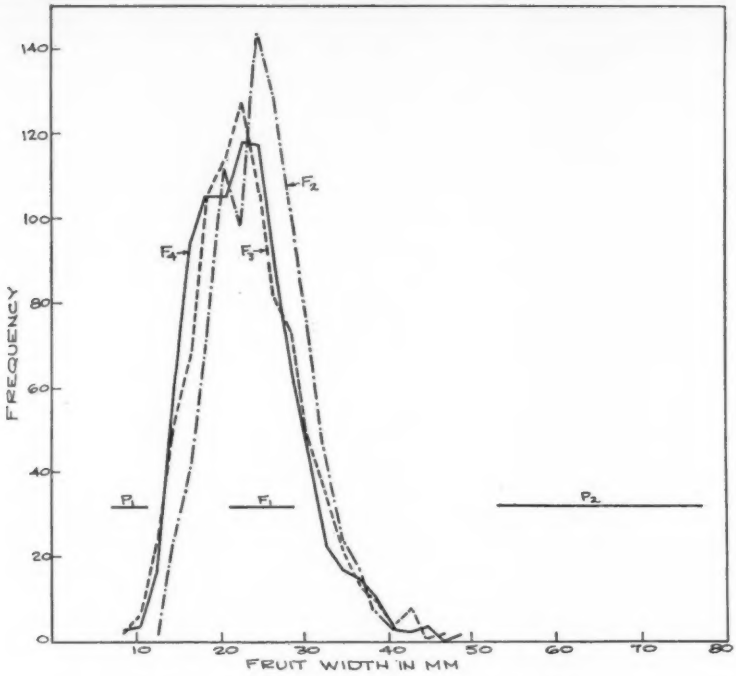


FIGURE 4.—Frequency distributions of logarithms of green fruit weights within generations.

dominance is of large size, but, by logarithmic transformation, the F_1 becomes larger than the F_2 mean as expected.

All evidence strongly points to a conclusion that the effects of genes and environment are multiplicative. It is necessary, therefore, to transform the original data to logarithms. After transformation, the frequency curves become normalized, except those of P_2 and F_1 which are negatively skewed. The trend of means from F_1 to F_4 decreases from 1.771 to 1.673; consequently, it may be inferred that there preponderantly exist genes with dominance or epistasis of large fruit weight. But the shift is away from the average of parental values (1.791), instead of toward it. An explanation may be offered that the size genes are favored in the genetic substrates of parental populations. It is obvious that any conclusion drawn from P_1, P_2, and F_1 values would be a misconception. Since F_1 (1.771) is smaller than $(P_1+P_2)/2=1.791$, one would assume dominance of small size, which contradicts the F_1, F_2, F_3, and F_4 results. Had not the data been transformed to logarithms, the contradiction would be much worse.

For purposes of analysis, it may be assumed that dominance of large fruit causes the decreasing trend of means of generations, for epistatic effects of quantitative genes are yet to be demonstrated. Indeed, some investigators

have shown no epistatic interaction of quantitative genes. In Drosophila egg production, STRAUS (1942) fails to find any interaction between genes on the three large chromosomes.

Ignoring epistasis, the excess of dominance of large size, $\sum d_i$, in logarithms, may be calculated for predicting means of the next unselected selfed generations.

$$\sum d_i = 4(F_2 \text{ mean} - F_3 \text{ mean}) = 4(1.758 - 1.694) = .256$$
$$\sum d_i = 8(F_3 \text{ mean} - F_4 \text{ mean}) = 8(1.694 - 1.673) = .168$$
$$\text{Average} = \frac{.256 + .168}{2} = .212.$$

Table 4 shows that P_1 (.0046) is somewhat more variable than P_2 (.0030) and F_1 (.0029). If the average of P_1, P_2, and F_1 variances, (.0035), can be used to estimate environmental variation of F_2, F_3, and F_4, the genotypic variance then is the total variance minus the environmental variance, as shown in table 4. The ratios of genotypic variances of segregating generations are:

$$\frac{H(F_3) - H(F_2)}{H(F_2) - H(F_1)} = \frac{.0471 - .0245}{.0245} = .92$$
$$\frac{H(F_4) - H(F_3)}{H(F_3) - H(F_2)} = \frac{.0468 - .0471}{.0471 - .0245} = -.01.$$

TABLE 4

Statistics of fruit weight.

	df	ARITHMETIC ANALYSIS					LOGARITHMIC ANALYSIS	
		MEAN	SKEWNESS g_1	MEAN	SKEWNESS g_1	MEAN SQUARE	GENOTYPIC VARIANCE	GENETIC VARIANCE
		gm		log				
Blocks	14					.0266		
Error	42					.0144		
P_1 (Red Chili)	285	9.0	0.62**	0.948	−0.26	.0046		
P_2 (Sunnybrook)	283	434.9	−0.12	2.634	−0.35*	.0030		
F_1	285	59.6	−0.12	1.771	−0.62**	.0029		
F_2	885	61.7	1.00**	1.758	0.13	.0281	.0245	.0175
F_2 total	883	56.3	1.40**	1.694	−0.15	.0506	.0471	1.50 (.0175)
between progenies	59					.4082		
within progenies	824					.0250	.50 (.0245)	.50 (.0175)
F_4 total	882	53.7	1.62**	1.673	−0.07	.0503	.0468	1.75 (.0175)
between progenies	59					.3940		
within progenies	823					.0257	.25 (.0245)	.25 (.0175)

$Sg_1 = \pm .14$ for P_1, P_2, and F_1; $Sg_1 = \pm .08$ for F_2, F_3, and F_4.

* Significant 1:20.
** Significant 1:100.

Logarithmic Analysis:

Average environmental variance = .0035.

Genotypic variance = Total variance—Environmental variance of .0035.

Regression of F_4 progeny means on F_3 progeny means = .64.

Genetic variance between F_3 progeny means $= .64\left(\frac{.4082}{15}\right) = .0175$.

Genotypic variance between F_3 progeny means = 1/15 (.4082–.0250) = .0256.

Genotypic variance between F_4 progeny means = 1/15 (.3940–.0257) = .0246.

If linkage and epistasis are absent, and if dominant deviation is d_i,

Variance of F_2 − Variance of F_3 progeny means

$$= 3/16 \sum d_i^2 = .0245 - .0256$$

$$\sum d_i^2 = -.0054.$$

Variance of F_3 progeny means − Variance of F_4 progeny means

$$= 3/64 \sum d_i^2 = .0256 - .0246$$

$$\sum d_i^2 = .0212.$$

Since $\sum d_i^2$ (−.0054) turns negative in the first comparison, there must be some unidentified phenomena counteracting dominant deviations or decreasing the variance. The fact that the values become positive (.0212) in the second test indicates that the effects of these unknown factors become lessened in advanced generations of selfing. In the computation, the independence of genes is assumed. It is clear that linked genes in repulsion phase would give smaller variance than the same genes not linked, and linkage effects slowly disappear as selfing continues. Thus, linkage, and also certain kinds of epistasis, may be used to explain the observed results.

Heritability, as measured by regression of F_4 on F_3, is .64. The genetic variance between F_3 progeny means is .64(.4082/15) = .0175 which equals genetic variance of F_2. Suppose genes are not linked and have equal effects, and the parental values can be used as the genetic range (1.686),

$$\text{Minimum number of genes} = \frac{(1.686)^2}{8(.0175)} = 20, \text{ which is an underestimate since}$$

parental values need not be the extreme genotypes of the range.

With the variance-ratio method, assuming no epistasis or linkage,

$$\text{"Effective" number of genes} = \frac{1/2(.0245)}{.000367} = 33, \text{ using } F_3 \text{ data,}$$

$$= \frac{1/4(.0245) \times 3}{.000256} = 72, \text{ using } F_4 \text{ data.}$$

The variance of variance (.000367 and .000256) may not be accurate since the design of experiment has been such that genotypes within progeny are confounded with block variation. Also, the estimation ignores linkage and epistasis, which seem to be operative in this character. If the assumption is not true, an error would be larger with the use of F_4 than F_3 values. Therefore, 33 is preferred to 72 as an estimate of number of genes. However, as the attempt is to calculate the minimum, the actual number may be much larger than 33.

DISCUSSION

Fruit Length and Width

Results of statistical analysis favor the hypothesis that mature fruit length

and width in the red pepper are primarily determined by shape and weight genes. As a consequence, from the breeder's viewpoint, it will then be impossible to find a segregating plant with fruit length and width beyond the limits of shape manifestation if weight is held constant. Length and width factors, *per se*, even if they are present, will provide only minor variations in the hybrids after shape effects have been established. The extremely transgressive segregation of length alone can hardly be genetically explained. Correlation studies also lead to the same conclusion that fruit length and width factors are transmitted together as shape.

Fruit Shape

The ratio of length and width was used as a shape index. Since environmental variations are multiplicative in P_1, P_2, and F_1, it is believed, by parallelism, that shape genes also act multiplicatively; thus, the values of shape indices were transformed to logarithms for analysis.

Trimodal distributions and apportionments of frequencies of F_2, F_3, and F_4 indicate a single gene pair for shape with incomplete dominance of oblate type. By assigning modal values to genotypes, oblate (OO) = .06 (index 1.15), heterozygote (Oo) = .18 (index 1.51), and elongated (oo) = .54 (index 3.43), it was discovered that the total observed genotypic variances are all accounted for by the segregation of this gene pair. The discrepancy between parental types and homozygous genotypes may be ascribed to expressivity of the gene in dissimilar genetic substrates and environmental conditions, which make selection of individual segregants for parental types difficult. With the regression method, heritability of F_3 progeny means was found to be 68 percent for fruit shape.

Fruit Weight

The distributions of fruit weights of F_2, F_3, and F_4 are unimodal and positively skewed, but, by transformation to logarithms, they become normalized. Using variances and trends of means as criteria, the environmental variation and genic manifestation of fruit weight were found to be multiplicatively cumulative. The inference is in accord with several investigators who, relying on parental averages, have observed the F_1, F_2, and backcross values to be closer to geometric than arithmetic means. But a contradiction between the two methods would arise with regard to dominance relations as is revealed in the results here. The definite decreasing trend of means of segregating generations will lead to a conclusion that genes are dominant for large or epistatic for large size even though the F_1 is lower than the average of parental values, from which dominance of small size is usually inferred. An argument has been presented that parental and F_1 values are not always dependable for making judgment on properties of genes because genetic substrates in those populations differ from each other.

Weight is the final fruit size attained by growth and development. Weight genes then must be those physiological factors which govern cell division and expansion, of which any variation would be multiplicative. So it is natural to find weight genes manifesting multiplicative action, which, according to

recent reports, seems to prevail in many quantitative characters which have been studied. In those characters, a proportionate progress in breeding would be a consequence. Selection for high values would be enhanced by the multiplicative actions of genes.

Results show that 64 percent of variance of F_3 progeny means for fruit weight is genetic. The minimum number of genes for weight differences is of the order of 20 to 33. The prevalent dominance is that of large size. With the observed variances, there are indications that some genes are linked in repulsion phase or are epistatic with each other, but no conclusive proof can be given in the present study.

SUMMARY

For the determination of number and nature of quantitative genes, fruit-size characters, as expressed by length, width, shape index (ratio of length to width), and weight, of the red pepper were chosen. Crosses were made between two varieties, the Red Chili (P_1) with small elongated fruit and the Sunnybrook (P_2) with large oblate fruit; and, without selection, the hybrids were selfed to the fourth filial generation. The parents and all the hybrids were tested in the same year. The data were subjected to statistical analysis and the estimates then compared with genetic expectations based on various types of gene action.

1. Results confirm the supposition that length and width of fruit are largely expressions of shape and weight factors. Evidences show the existence of specific genes for shape. The presence of genes for length and width *per se* is unlikely; and even if they are present, they would cause only small deviations after shape manifestations have been accounted for.

2. Trimodal logarithmic distributions of segregating generations for fruit shape fit a one-gene hypothesis. When modal values are used to evaluate genotypes, the oblate (*OO*) shape index is 1.15 (log. .06), the heterozygote (*Oo*) 1.51 (log .18), and the elongated (*oo*) 3.43 (log. .54), oblate being partially dominant to elongated. The variances contributed by this gene pair alone approximate the observed genotypic variances. Sixty-eight percent of fruit-shape differences of F_3 progeny means is genetic.

3. Distributions of fruit weights of F_2, F_3, and F_4 are positively skewed, but become normalized by logarithmic transformation. The effects of genes and environment on fruit weight are found to be multiplicative. The minimum number of genes in this pepper cross has been estimated to be between twenty and thirty-three. The decreasing trend of means of selfed generations suggests that the genes are preponderantly dominant for large or epistatic for large weight. A study of variance reveals some evidences of linkage and epistasis. Heritability of F_3 progeny means is 64 percent.

ACKNOWLEDGEMENT

The author gratefully acknowledges the encouragement, advice, and help given by the late DR. ERNEST WALTER LINDSTROM, under whom this investigation was conducted.

LITERATURE CITED

CASTLE, W. E., 1921 An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. Science **54:** 223.

CHARLES, D. R., and H. H. SMITH, 1939 Distinguishing between two types of gene action in quantitative inheritance. Genetics **24:** 34–48.

GUSTAFSSON, A., 1946 The effect of heterozygosity on variability and vigor. Hereditas **32:** 263–286.

HUTCHINS, A. E., 1934 Morphological relationships in the ontogeny of the cultivated cucumber, *Cucumis sativus* L. Minn. agric. Exp. Sta. Tech. Bull. **96:** 35 pp.

KAISER, S., 1935 The factors governing shape and size in *Capsicum* fruits; a genetic and developmental analysis. Torreya Bull. **62:** 433–454.

LINDSTROM, E. W., 1927 Linkage of size, shape, and color genes in *Lycopersicum*. Verh. V. int. Kongr. Vererb., Berlin **2:** 1031–1057.

1946 Genetic relations of inbred lines of corn. Iowa agric. Exp. Sta. Rep. agric. Res. 1945–1946. Part II. 43–45.

PANSE, V. G., 1940 Application of genetics to plant breeding. II. The inheritance of quantitative characters and plant breeding. J. Genet. **40:** 283–302.

POWERS, LEROY, 1942 The nature of the series of environmental variances and the geometric means in crosses involving species of Lycopersicon. Genetics **27:** 561–575.

SHULL, G. H., 1921 Estimating the number of genetic factors concerned in blending inheritance. Amer. Nat. **55:** 556–564.

SINNOTT, E. W., 1935 Evidence for the existence of genes controlling shape. Genetics **20:** 12–21.

SINNOTT, E. W., and C. B. DURHAM, 1929 Developmental history of the fruit in lines of *Cucurbita pepo* differing in fruit shape. Bot. Gaz. **87:** 411–421.

STRAUS, F. S., 1942 A genetic mechanism of heterosis as demonstrated by egg production in *Drosophila*. Unpublished Ph.D. Dissertation. Iowa State College, Ames, Iowa.

WEETMAN, L. M., 1937 Inheritance and correlation of shape, size, and color in the watermelon. Iowa agric. Exp. Sta. Res. Bull. **228:** 222–256.

WRIGHT, S., 1934 The results of crosses between inbred strains of guinea pigs, differing in number of digits. Genetics **19:** 537–551.

YEAGER, A. F., 1937 Studies on the inheritance and development of fruit size and shape in the tomato. J. agric. Res. **55:** 141–152.

MICROSCOPICALLY VISIBLE BODIES IN THE CYTOPLASM OF THE "KILLER" STRAINS OF PARAMECIUM AURELIA[1]*

JOHN R. PREER, JR.

Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania

Received November 18, 1949

KILLER strains of *Paramecium aurelia* possess the cytoplasmic factor kappa and liberate one of a series of antibiotics called paramecins into the fluid in which they live. Sensitive strains lack kappa and are killed by paramecins. Each killer strain is resistant to the paramecin which it produces. Cytological studies have been undertaken for the purpose of staining the cytoplasmic factor kappa and studying its characteristics microscopically. Preliminary papers concerned with these studies (PREER 1948b, c) reported the finding of stainable bodies in killers which were identified with kappa. The cytological techniques, the characteristics of the bodies, and the results of experiments which indicate the identity of the bodies and kappa are reported here in detail.

SONNEBORN (1943), working with variety 4 of *P. aurelia*, found that killers possess the cytoplasmic factor kappa and the dominant gene *K*, in whose presence kappa is perpetuated. Kappa is not perpetuated in animals homozygous for the recessive gene *k*, and such animals are sensitives. The dominant gene *K* cannot initiate the production of kappa, and animals containing *K* but lacking kappa are also sensitives. By transfer of cytoplasm at conjugation, kappa can be carried from killers to sensitives lacking kappa but having the gene *K*; in these animals kappa increases and the sensitives become killers. SONNEBORN (1948) found that kappa may also be transferred by exposing sensitives (lacking kappa but having the gene *K*) to high concentrations of broken up killer animals.

PREER (1946, 1948a) found that in variety 2 of *P. aurelia* the multiplication rate of the animals may be made to exceed the rate of increase of kappa by providing the animals with a large daily supply of food. The number of particles of kappa per animal decreases until finally it reaches zero in some individuals. When animals having a reduced number of particles (but not completely lacking them) are made to reproduce slowly by limiting the daily food supply, the number of particles of kappa increases faster than the number of animals until the original concentration is restored. These changes in the concentration of kappa result in changes in the killer character. Animals with a somewhat reduced kappa concentration are weak killers, producing little paramecin; while animals with few or no particles are sensitive to paramecin. Animals which are completely freed of kappa remain permanent sensitives under all conditions. By taking advantage of this relation between the rate of increase of kappa and the rate of increase of the animals the author (1)

[1] Aided by a grant from the DONNER FOUNDATION and PHI BETA PSI SORORITY.

* The cost of the accompanying figures is paid by the GALTON AND MENDEL MEMORIAL FUND.

demonstrated the capacity of a single particle to restore, under appropriate conditions, the normal kappa concentration; (2) estimated that a strong killer has approximately 450 or possibly a somewhat larger number of kappa particles; and (3) measured the rate of increase of the particles under different conditions.

Studies on the effects of X-rays on killers (PREER 1948b) revealed that kappa may be destroyed by radiation and the particle number decreased until animals are completely freed of all kappa. The number of particles in a strong killer was estimated from radiation data to be 400–1600. Kappa was reported to have an inactivation dose typical of bacteria (3500 roentgens) suggesting that the size of kappa may be comparable to that of bacteria.

Since this last fact suggests that kappa may be large enough to be microscopically visible, a cytological study was undertaken to see if particles agreeing with the above described properties of kappa could be demonstrated. The detailed account of the finding of such particles will now be given.

MATERIALS AND METHODS

Twenty-two of SONNEBORN'S and HARRISON'S stocks of *P. aurelia* have been used in this study. Each stock consists of the descendents of a single individual isolated from a stream or pond. A list of the stocks and their sources follows: D from Counterfeiter's Ledge, N. Y.; E from Elkridge, Md.; G from near Pinehurst, N. C.; H from Halethorpe, Md.; K from Johns Hopkins University Botanical Gardens, Baltimore, Md.; U from Woodstock, Md.; W from Williamstown, Mass.; 28 from Woods Hole, Mass.; 30 from Coates Pond, Md.; 36 from Hamden, Conn,; 50 from an unknown locality in Ore.; 51 from Spencer, Ind.; 53 from Twin Lakes, near Bloomington, Ind.; 54 from Flatrock Creek, Moscow, Ind.; 61–2 from Millbrook, N. J.; 63–2 from Blairstown, N. J.; 64–1 from Blairstown, N. J.; 66–3 from Millbrook, N. J.; 75–1 from Jenkintown, Pa.; 81–1 from Huntington Valley, Pa.; 117–3 from Andover, N. J.; 308–2 from Jacksonville, Pa. All of these stocks belong to variety 2 of *P. aurelia* except stock 51, which belongs to variety 4. (See SONNEBORN and DIPPELL, 1946 for an account of the varieties of *P. aurelia*.) One stock of *P. caudatum* was used, 79a from Philadelphia, Pa.

Stocks G, H, 36, 50, 51, 308–2, and a variant of G called Gml are killers. The characteristic action of each of these killer stocks on sensitives has been described (SONNEBORN 1943, PREER 1948a) with the exception of stock 308–2. Its action is much like that of stock H, but is very much weaker. The remaining stocks are non-killers. Stock 79a of *P. caudatum* was used as a standard sensitive because of its great sensitivity to all the killers and because of the fact that it can be easily distinguished from *P. aurelia* in mixtures by its larger size.

Methods of culture and control of fission rate were similar to those reported in detail by SONNEBORN and DIPPELL (1946) and PREER (1948a) except that *Aerobacter cloacae* was used instead of *A. aerogenes* as a food organism.

X-radiation was carried out in thin cellulose acetate boxes about one centi-

meter square and three millimeters deep, with a depth of fluid containing paramecia of about two millimeters. All radiation was given at 13 cm with no filter at 200 kv and 15 milliamps; this gave a rate of about 2400 roentgens per minute.

Cytological techniques were as follows. Fixation was accomplished by forcefully pipetting about one ml of a concentrated suspension of paramecia into about five ml of Schaudinn's fixative in a 15 ml centrifuge tube at 50°C. The Schaudinn's fixative was prepared fresh just before use by mixing 20 ml saturated aqueous $HgCl_2$, 10 ml 95 percent ethyl alcohol, and 1.5 ml glacial acetic acid. As quickly as possibly after the animals were introduced into the centrifuge tube containing the fixative, the tube was filled to 15 ml with 95 percent ethyl alcohol, the animals centrifuged down, the fixative and alcohol decanted, and 15 ml of fresh 95 percent alcohol added. By means of additional centrifuging and decanting animals were passed through absolute ethyl alcohol and finally suspended in one or two ml of xylol. The animals were pipetted onto an albumen-smeared slide where they were allowed a few seconds to settle and adhere to the albumen. Slides were then placed into coplin jars containing xylol. Animals placed on slides from xylol in this manner were found to be firmly attached and very few were lost in the subsequent hydrolysis and staining procedures.

Two staining methods were used extensively. The first of these was the Feulgen technique as given by RAFALCO (1946). Cytoplasmic particles were revealed by the Feulgen technique only under optimal conditions of staining intensity.

In the second staining method animals were hydrolyzed just as in the Feulgen technique, but animals were then stained overnight in Giemsa stain according to a modification of the method described by WOLBACH (1919). The procedure was as follows: Pass slides from xylol through the alcohols to water; cold N HCl, two minutes; N HCl at 60°C, ten minutes; wash in distilled water thoroughly. Stain overnight in 1.25 ml liquid Giemsa stain in 100 ml distilled water plus 2.5 ml absolute methyl alcohol. Destaining was carried out by alternately placing slides in running distilled water for a few seconds then a mixture containing 5 percent xylol and 95 percent acetone for a few seconds. This passage of slides between the flowing water and acetone-xylol mixture was repeated until animals were properly destained—usually three or four passages. Progress of destaining was observed under low magnification after animals had been dehydrated and placed into xylol. Dehydration was accomplished by passing slides quickly through a succession of xylol-acetone mixtures: 5 percent xylol in acetone, 25 percent xylol, 50 percent xylol, 75 percent xylol, xylol. If differentiation was incomplete when slides were examined in xylol, they were carried through the xylol-acetone series for more passages between flowing water and xylol-acetone. This whole procedure was repeated until differentiation was satisfactory. The method served to bring out not only the cytoplasmic bodies but also was an excellent stain for nuclei, trichocysts, and bacteria in food vacuoles.

CYTOLOGICAL OBSERVATIONS

Killers stained by the Feulgen technique showed numerous very small and faint Feulgen positive bodies scattered through the cytoplasm outside of the food vacuoles. The bodies stained very intensely with Giemsa preceded by hydrolysis. They appear about the same size and shape after the Feulgen technique as they do after the Giemsa stain. They differ in the different killer stocks, but in general, appear as minute, spherical to ovoid bodies, often double. Using a micrometer eyepiece it was estimated that single forms vary in size from 0.2–0.8 micron. Considerable error, of course, may be involved in the estimation by this method of the size of bodies so small.

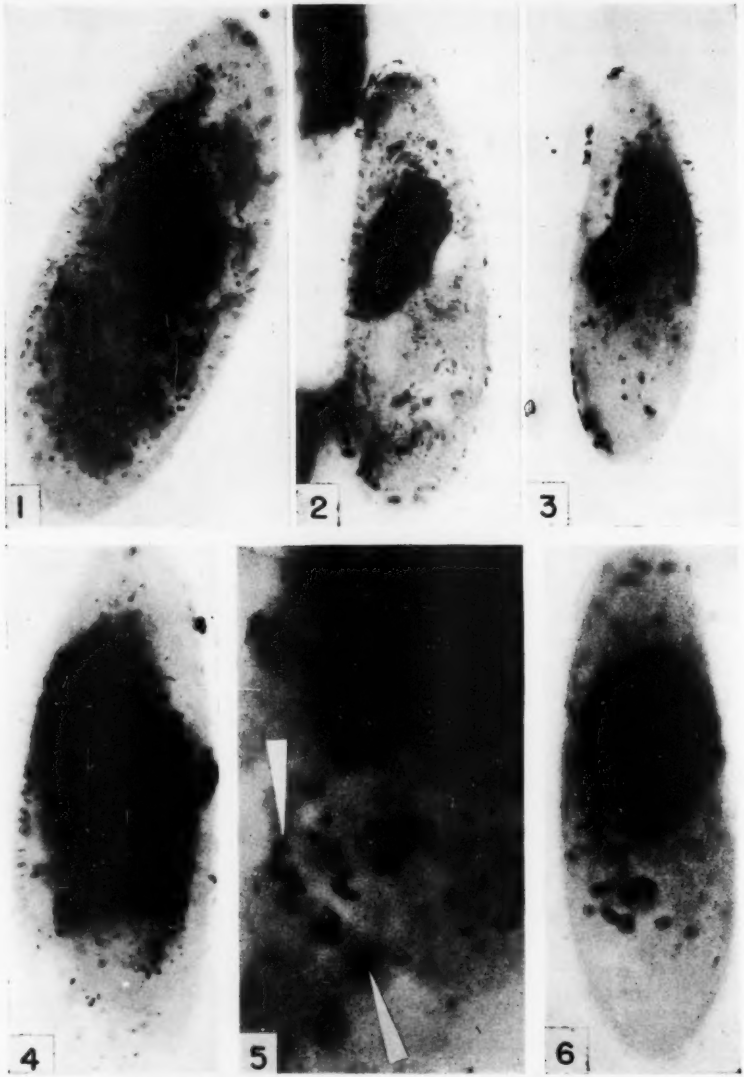
Photographs of animals stained with Giemsa are illustrated in figures 1–6. Figure 1 shows a stock G killer. The cytoplasmic bodies are rather uniform in appearance with single forms being estimated at about 0.4 micron in length, slightly less than this in width. Figure 2 shows killer Gml, which has rather uniform but somewhat smaller bodies, estimated at about 0.2 to 0.3 micron in length. Killer stock 36 (not shown) resembles these two killers in the uniformity of bodies, but the bodies are slightly larger (0.5 micron). The bodies within each of the remaining killer stocks (H, 50, 308–2, and 51) are much less uniform. Stock 51 is shown in figure 3. The bodies in H and 50 are much like those in 51. Sizes in each of these stocks range from bodies as small as those in Gml up to bodies 0.8 mm in diameter. Shape is also variable, ranging from rod-like to spherical. Stock 308–2 (figure 4) is characterized by having perhaps the smallest as well as the largest bodies to be found in any of the killers. It is also characterized by numerous double forms consisting of a large sphere and a very small sphere with a rather lighter area or a space in between (see figure 5). It also possesses the usual large and small double forms in which the two parts are of more nearly the same size.

No Feulgen positive or Giemsa staining bodies have been found outside the food vacuoles in the non-killers, except for occasional relatively rare particles of various shapes and sizes; these are presumably crystals or other foreign matter. (Cf. p. 353) An animal of a sensitive strain of stock G is shown in figure 6. The following non-killer stocks have been examined: D, E, K, U, W, 28, 30, 53, 54, 61–2, 63–2, 64–1, 66–3, 75–1, 81–1, 117–3, 79a.

EVIDENCE FOR IDENTIFYING THE BODIES WITH KAPPA

Six lines of evidence indicate that kappa and the bodies are identical: (1) presence of stainable bodies in killers and absence in non-killers, (2) agreement in number of kappa particles and stainable bodies in strong killers, (3) parallel in concentration of the two during experimental variation of their concentration, (4) agreement in size, (5) agreement in probable chemical constitution, and (6) agreement in probable mode of increase. Each one of these points will now be considered.

1. The bodies were found in all killers but in none of the non-killers examined. This is true not only of the naturally occurring stocks but also of the non-killer strains produced from killers in the laboratory. Thus, non-killer



FIGURES 1–6.—Paramecia were fixed in acetic Schaudinn's, hydrolyzed with N HCl for ten minutes at 60°C and stained with Giemsa stain. Numerous stained bodies outside the food vacuoles are seen throughout the cytoplasm in the killers (fig. 1–5). Sensitive (fig. 6) lacks such bodies. Figure 1.—Killer stock G. ×765. Figure 2.—Killer strain Gml. ×765. Figure 3.—Killer stock 51. ×765. Figure 4.—Killer stock 308–2. ×765. Figure 5.—Killer stock 308–2. ×1530. Arrows point to double forms with the two parts of unequal size. Figure 6.—Sensitive strain G. ×765. Group of stained bodies in lower part of cytoplasm are partially digested bacteria in a food vacuole.

strains of G, H, 36, and 50 derived from killers by rapid multiplication contained no stainable cytoplasmic bodies. This association of killers and bodies was shown clearly in the following experiment in which killer cultures were radiated sufficiently to destroy all of the kappa in some, but not all, animals in the cultures. It was found that the particular animals which were freed of all kappa gave rise to non-killer cultures lacking stainable bodies, while in the same radiated cultures the animals retaining some kappa gave rise to killer cultures possessing stainable bodies.

Experiment No. 1: Two portions of a culture of killers of stock G were treated with X-rays. The first portion received 24,000 r and the second portion received 28,000 r—doses known to destroy most, but not all, of the kappa in the killers. Thirty-six animals from each of the two series were isolated into individual containers, cultured slowly by restricting the food supply (one-half fission per day) to allow the concentration of kappa to rise and produce killers in the cultures derived from animals in which all of the kappa had not been destroyed. Killing tests after two weeks revealed that 5 out of 28 surviving cultures (18 percent) which received 24,000 r contained only sensitive non-killers and 29 of the 32 surviving cultures (91 percent) which received 28,000 r contained only sensitive non-killers. These cultures were thus derived from animals which had been freed of all kappa particles. The remaining cultures consisted of killers, and hence were derived from animals which had not been completely freed of kappa. The five sensitive cultures and seven of the killer cultures from the 24,000 r series, and the three killer cultures and nine of the sensitive cultures from the 28,000 r series were stained. Numerous stainable bodies typical of stock G were found in the animals from all of the kappa-containing killer cultures and no bodies were found in animals from the kappa-free sensitive cultures. Since chance alone determined which particular animals were freed entirely of kappa by the X-rays and since precisely the same animals losing all kappa also lost the stainable bodies, it is concluded that there is an extremely close relation, if not identity, between the two.

2. Agreement has been found between number of particles of kappa and number of stainable bodies in killers. In giving this evidence for identity of kappa and stainable bodies, we will consider: (a) estimates of the number of particles of kappa made on the basis of differential multiplication rate studies; (b) estimates of the number of particles of kappa made on the basis of X-ray inactivation studies; and (c) direct counts of the number of stainable bodies in strong killers.

The method of estimating the number of kappa particles by differential multiplication has been described (PREER 1948a) and two experiments were cited which provided data for calculations of particle number. In the first of the experiments animals of stock G multiplied at the rate of 3.4 fissions per day while it was calculated that kappa increased at the rate of 2.0 doublings per day; the number of particles of kappa per strong killer was estimated at 440. In the second experiment the animals multiplied at 2.6 fissions per day; kappa increased at 2.1 doublings per day; and the particle number was estimated as 460. Because of the assumptions on which the method was based,

these estimates of particle number were considered minimum values. In a more recent experiment, an account of which follows, the animals multiplied at 3.5 fissions per day; it was calculated that kappa increased at 2.1 doublings per day and that the particle number was estimated at 1400.

Experiment No. 2: 40 killers of stock G were isolated and allowed to multiply at 3.5 fissions per day at 27°C. At intervals, animals were isolated and cultured slowly to allow the concentration of kappa to rise in the progeny of those animals still possessing kappa particles. Later, tests for killers were made to determine what proportion of the originally isolated animals had given rise to exclusively non-killer progeny, i.e., those of which had been freed of kappa. The results are given below:

MEAN NUMBER OF FISSIONS (n)	NUMBER OF LINES OF DESCENT		PROPORTION FREED OF KAPPA (W_n)
	LACKING KAPPA	TOTAL	
12	0	103	0.00
15	1	100	0.01
17	7	101	0.07
19	10	102	0.10
22	40	99	0.40
25	72	98	0.73
27	80	93	0.86
30	81	83	0.98

Calculations of the rate of kappa increase and particle number according to methods previously employed (PREER 1948a) gave a rate of kappa increase of 2.1 doublings per day and a particle number of 1400.

The number of kappa particles estimated on the basis of radiation studies will now be considered. It has been reported (PREER 1948b) that the number of particles of kappa per animal as determined by X-radiation was between 400 and 1600. However, more precise methods of treating the data on which those estimates were based and the acquisition of new data give three calculations of particle number: 610 in one experiment, 1100 in a second, and 320 in a third. An account of these three experiments and methods of calculation follows.

Experiment No. 3: Cultures of killers of stock G were radiated with different doses of X-rays and from each culture 108 isolations were made. For three weeks the isolation cultures were fed sufficient food to allow an average fission rate of about 0.3 to 0.5 fissions per day in order to enable surviving kappa particles to increase. Considerable mortality not associated with characteristic killer activity was encountered in some cultures particularly toward the end of the period, and cultures in which dead animals were found were discarded. At the end of this period 20 to 50 individuals of the sensitive stock of *P. caudatum*, 79a, were added. Observations for killing activity of the cultures were made on the next successive three days. Preliminary experiments had shown that any cultures which failed to show killing activity when tested in this manner three weeks after radiation did not develop killing activity during more extended periods of slow multiplication. The number of cultures con-

sisting of exclusively non-killer animals (and thus the number of X-rayed animals freed of kappa; PREER 1948b) are given below:

DOSE IN ROENTGENS (D)	NUMBER OF LINES OF DESCENT		PROPORTION FREED OF KAPPA (W_D)
	LACKING KAPPA	TOTAL (N)	
12,200	0	104	0.00
16,300	0	105	0.00
20,400	32	104	0.31
24,500	71	98	0.72
28,600	85	98	0.87
32,700	91	93	0.98
36,800	76	76	1.00
40,900	93	93	1.00

Experiment No. 4: This experiment was performed exactly as the preceding. Results are as follows:

DOSE IN ROENTGENS (D)	NUMBER OF LINES OF DESCENT		PROPORTION FREED OF KAPPA (W_D)
	NUMBER FREED OF KAPPA	TOTAL NUMBER (N)	
17,000	0	100	0.00
19,400	5	90	0.06
21,800	8	91	0.09
24,300	49	99	0.49
26,700	74	97	0.74
29,100	73	97	0.75
31,500	86	90	0.96
33,900	89	92	0.97
36,400	85	88	0.97

Experiment No. 5: This experiment was performed just as the preceding two.

DOSE IN ROENTGENS (D)	NUMBER OF LINES OF DESCENT		PROPORTION FREED OF KAPPA (W_D)
	NUMBER FREED OF KAPPA	TOTAL NUMBER (N)	
15,000	0	76	0.00
17,500	0	78	0.00
20,000	0	74	0.00
22,500	27	66	0.41
25,000	64	83	0.71
27,500	13	18	0.72
30,000	60	82	0.73
32,500	74	79	0.94
35,000	71	75	0.95
37,500	75	77	0.97
40,000	74	75	0.99

Calculations of the initial number of particles per animal were made as follows. First, assume that after radiation the surviving particles are distributed among the animals in a Poisson distribution. It can be shown that this condition will be approximated if (1) the probability of surviving the radiation is the same for each of the initial particles, and (2) if prior to radiation the particles are approximately equal in number in the animals, or are randomly distributed among the animals. Then let $\bar{x}$ equal the mean number after dose D and W_D equal the probability of an animal having no particles after dose D. The first term of the Poisson distribution will be

$$W_D = e^{-\bar{x}}. \tag{1}$$

When the values of $\bar{x}$ for the three experiments were calculated by use of this relation, and plotted logarithmically against dose, it was noted that they fitted straight lines fairly well. A linear relation of this type is predicted by the target theory in the case of one-hit inactivations. The lines may be expressed by

$$\ln \bar{x} = bD + a \tag{2}$$

where b and a are constants. Or, taking logarithms of (1) and combining (1) and (2) we may write

$$\ln (-\ln W_D) = bD + a. \tag{3}$$

Thus a linear relation should be obtained if $\ln(-\ln)$ of the values of W_D is plotted against dose. If the linear relation is assumed to hold at lower doses, the lines may be extrapolated back to zero dosage to obtain the initial value of W_D, and from that, by use of equation (1), the initial number of particles. Furthermore, the inactivation dose, or dose corresponding to 37 percent survival of particles, can also be obtained from the extrapolated curve.

The data of the three experiments were fitted separately to equation (3) using the method of least squares and using appropriate weights. The curves are given in figures 7, 8, and 9. The weights used in fitting the data should be the reciprocal of the variance of the values of W_D, which is $N/W_D(1-W_D)$ (where N is the total number of cultures on which each value of W is based). Because of the logarithmic transformation, this weight must be multiplied by the reciprocal of the square of the derivative of $\ln(-\ln W_D)$, or $(W_D \ln W_D)^2$. This gives $NW_D(\ln W_D)^2/(1-W_D)$ as the appropriate weight which was used.

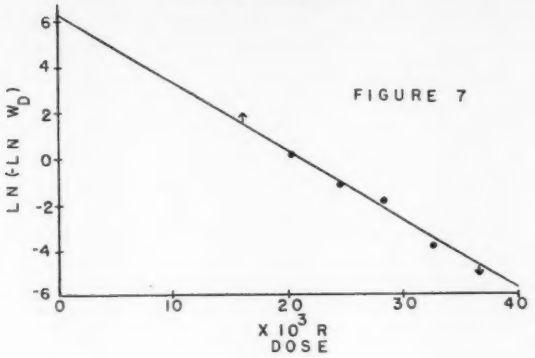
The question arose as to the proper procedure in those cases where according to the data W_D apparently had the value zero or one. Since it is evident that $0<W_D<1$, and since $\ln(-\ln W_D)$ in the case of W_D equal zero or one is infinite the points cannot be plotted. One way of handling the situation is to assign a weight of zero to such cases, i.e. disregard them. This was done, and the lines plotted according to the method outlined above. Extrapolation of the lines and calculation of expected numbers revealed that in some cases where W_D was zero according to the data a much higher value was expected. For example in Experiment No. 5 0 out of 78 kappa-free animals were found after 17,800 r. The expected number obtained by extrapolation of the fitted line was,

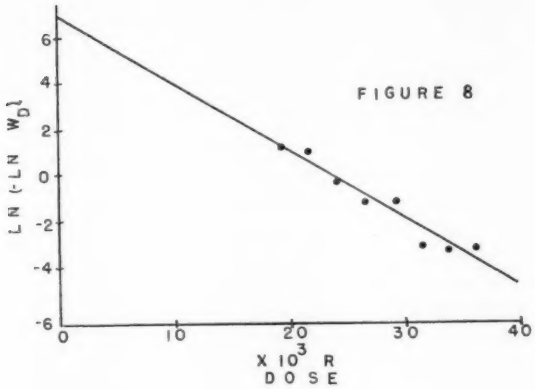
however, about 21 out of 78. Thus the fitted line based on weight zero for the ratio predicts that 21 out of 78 should be found and ignores the fact that none were found—clearly biasing the estimate.

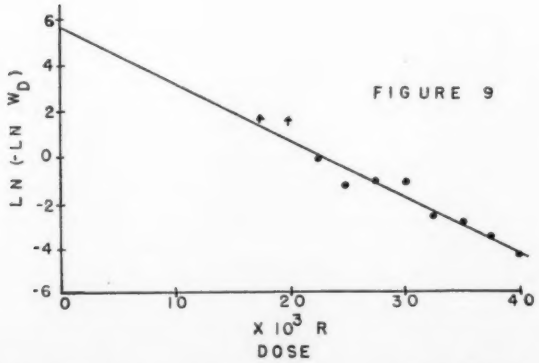
This bias was reduced by a procedure based on the following considerations. The use of enumeration data such as these, consisting of the number of animals freed of kappa divided by the total, are used to estimate the value of the continuous probability variable W_D, which cannot be zero or one for the case in question. If none of a series of 78 lack kappa, this does not mean that the best estimate of the probability W_D is zero; it indicates that as far as one can judge on the basis of the sample the best estimate is $0 < W_D \leqq 0.5/78$. If the curve which was fitted by using weight zero for this value had passed through this interval, then the finding of 0/78 would have been consistent with the other data as a whole and the best fit would have been obtained. Since the fitted curve indicated a probability greater than 0.5/78, the curve was refitted using the maximum sample estimate of W_D (0.5/78). The same reasoning and procedure was applied in cases where $W_D = 1$. This procedure of handling ratios of zero and one was followed in fitting all the lines. In figures 7, 8, and 9, a small arrow indicates the limiting value of those points which were used in obtaining the final fitted lines. For Experiment No. 3 the initial number of particles per animal was estimated at 610 and the inactivation dose was 3500 r. For Experiment No. 4 the initial number of particles per animal was 1100 and the inactivation dose was 3400 r. For Experiment No. 5 the number of particles was 320 and the inactivation dose was 4000 r.

Thus six independent estimates of the number of particles of kappa, three using differential multiplication rates and three using X-ray inactivation data, range from 320–1400. How do these determinations compare with the number of stainable bodies found in killers? On four different occasions killers of stock G growing at 0.4 fissions per day at 27°C were fixed, stained, and the number of bodies per animal were counted. By using an ocular insert giving a cross-hatched field, the counts were readily made. The presence of food vacuoles and the macronucleus undoubtedly served to make the counts somewhat too low by obscuring the bodies. The double bodies also presented another problem; double forms were arbitrarily counted as two if a clear space with no visible connections separated the two parts. The possibility of counting occasional stray granules or crystals not identical with the bodies found in killers was also considered. These were rare and unimportant as shown by a count of ten individuals of a kappa-free strain of stock G—counts ranged from zero to three per animal, with a mean of 1.2 ± 0.3. (The value following the plus or minus signs here and subsequently is the estimated standard error.) The results of counts on four different occasions were as shown at top of page 355. These counts of stainable bodies are in agreement with the number of particles of kappa. Further, they show a real variation in the number of bodies at different times. This probably accounts for some of the variation in the calculations of the number of kappa particles.

3. Further evidence for the identity of the bodies and kappa is found in the quantitative parallel in the two during experimental variation of their







FIGURES 7–9.—Ln(−ln W_D) (W_D is the proportion of animals freed of kappa) plotted against D (X-ray dose). See p. 353 for meaning of arrows. Figure 7 is based on Experiment No. 3, figure 8 based on Experiment No. 9, and figure 9 based on Experiment No. 10.

NUMBER OF ANIMALS COUNTED	NUMBER OF BODIES	
	MEAN ± STANDARD ERROR	RANGE
5	631 ± 61	400–738
6	1125 ± 42	1015–1310
5	1179 ± 54	1029–1277
5	794 ± 41	693–886

concentration. Rapid multiplication rate and X-radiation have been used to lower the kappa concentration, and experiments involving each of these two techniques will now be considered.

Strong killers containing many particles of kappa were allowed to multiply rapidly, thus lowering the number of particles per animal. Estimates of the number of particles of kappa after different numbers of fissions were compared with direct counts of the number of visible bodies. The data of Experiment No. 2 were used to calculate the mean number of kappa particles after successive fissions. These calculations were made by assuming logistic particle increase and making use of relations developed by OTTER and WRIGHT (PREER 1948a). The resulting mean numbers of kappa particles form a smooth curve when plotted logarithmically against fissions in figure 10.

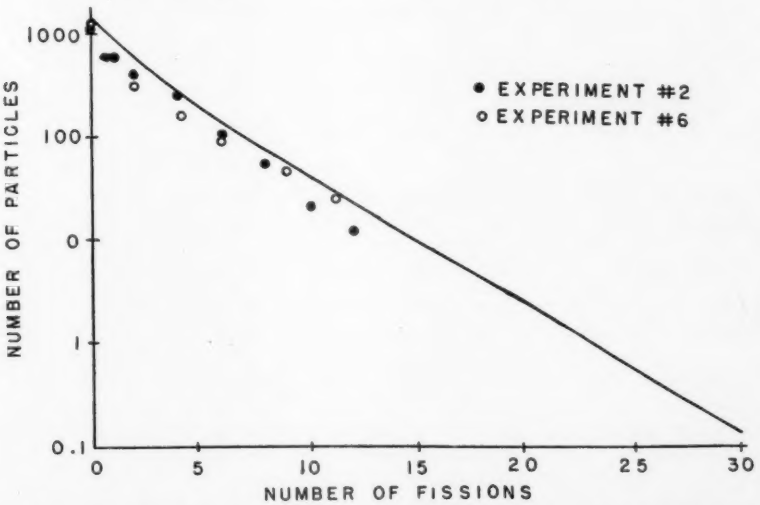


FIGURE 10.—Smooth curve represents number of kappa particles plotted logarithmically against fissions on the basis of Experiment No. 2. Solid and open circles are means of counts of stainable bodies.

The solid and open circles shown in figure 10 represent counts of stainable bodies from two different experiments. Data represented by the solid circles

were obtained from Experiment No. 2, the same experiment that yielded the data on which the curve representing kappa concentration was based. To obtain the counts of stainable bodies in this experiment, after different numbers of fissions, samples of animals were taken from the cultures, fixed, stained, and the number of visible bodies recorded. The results follow:

NUMBER OF FISSIONS	NUMBER OF ANIMALS COUNTED	MEAN NUMBER OF BODIES
0	6	1125±42
1	6	588±16
2	6	423±27
4	6	248±12
6	6	104±7
8	12	52±5
10	12	21±2
12	12	12±2

Counts were not extended to animals having a lower mean number, because of the lowered precision of counts in such cases. As already pointed out occasional bodies are found in kappa free animals which may be confused with the bodies associated with killers.

Counts of stainable bodies represented by the open circles in figure 10 were obtained in another experiment:

Experiment No. 6: This experiment was identical with Experiment No. 2 except stainable bodies alone, and not kappa particles were estimated.

Results follow:

NUMBER OF FISSIONS	NUMBER OF ANIMALS COUNTED	MEAN NUMBER OF BODIES PER ANIMAL
0	5	1179±54
2	4	313±45
4	11	156±17
6	12	96±10
9	12	47±5
11	11	24±3

These numbers of stainable bodies are plotted in figure 10 as open circles, and it is noted that they are in agreement with the closed circles based on the counts of Experiment No. 2. It is also noted that in figure 10 the points representing numbers of stainable bodies agree well with the general shape and slope of the curve for kappa, but that they fall consistently below the curve. This discrepancy may be due to the fact, as already mentioned, that counts of bodies are too low because many are obscured by the macronucleus and food vacuoles. On the other hand, when one considers the extreme nature of the extrapolation involved in computing the curve for kappa, a deviation of this magnitude is probably to be expected. (The curve for kappa is based on data

consisting of the number of animals freed of kappa between fissions 15 and 30, and the portion of the curve between 0 and 15 fissions represents an extrapolation). The agreement in shape and slope between the curve for kappa and the curve formed by the counts of stainable bodies suggests identity of the two.

Similar experiments using X-rays were attempted in order to compare the number of stainable bodies surviving different doses of radiation with the calculated numbers of kappa particles. A complicating factor in this investigation was shown in preliminary experiments in which it was found that animals fixed and stained immediately after radiation showed no reduction in the number of visible bodies. If some time intervenes between radiation and fixation, however, particles disintegrate. Thus when a culture was given 43,000 r, enough radiation to destroy virtually all the kappa, fixation and staining after 24 hours at 27°C showed very little reduction in number of bodies; staining after 48 hours, however, gave a count of only about three to five bodies per animal; counts made after 72 hours showed none to five. During this period animal multiplication was prevented by withholding food. Counts seemed to be somewhat higher than those on G non-killer strains and this might be attributed to the presence of still incompletely disintegrated bodies. The procedure adopted in investigating the number of stainable bodies surviving after different X-ray dosages was to radiate, hold cultures 72 hours in the dark at 27° without feeding, then fix, stain, and count.

Another error which might be introduced by this procedure is the possibility of increase of unaffected bodies during the 72 hour period allowed for disintegration. Cessation of animal multiplication brought about by withholding further feeding during the 72 hour period should tend to retard multiplication of bodies. However, any increase would make the counts somewhat too high especially for greater doses where the concentration of bodies is low and the possibility of many-fold increase exists. As will be shown presently, the data do, in fact, show counts somewhat higher than expected for high doses, and increase of bodies subsequent to radiation may account in part for this apparent discrepancy.

In Experiment No. 5 the proportions of animals freed of kappa by different doses of radiation were determined, and these data used to calculate the initial number of particles of kappa. In the same experiment the numbers of stainable bodies were ascertained 72 hours after radiation. Counts follow:

DOSE IN ROENTGENS	NUMBER OF ANIMALS COUNTED	MEAN NUMBER OF BODIES PER ANIMAL
0	5	795±41
2,500	5	560±11
5,000	5	321±26
7,500	5	293±12
10,000	5	226±14
12,500	5	117±12
15,000	8	27±5
17,500	9	23±4
20,000	10	9±2

Experiment No. 7: This experiment was similar to the preceding except stainable bodies alone, and not kappa were estimated. Results follow:

DOSE IN ROENTGENS	NUMBER OF ANIMALS COUNTED	MEAN NUMBER OF BODIES PER ANIMAL
0	5	631±60
2,500	4	551±86
5,000	4	392±57
7,500	4	299±64
10,000	4	219±43
12,500	4	131±43
15,000	4	47±8
17,500	4	22±6
20,000	4	22±5

The mean number of stainable bodies counted in these two experiments are plotted in figure 11, open circles for Experiment No. 5 and open squares for Experiment No. 7. The mean number of kappa particles ($\bar{x}$) calculated from the proportion of animals freed of kappa (W_D) in Experiment No. 5 are also plotted in figure 11, the points being designated by closed circles. Use was made

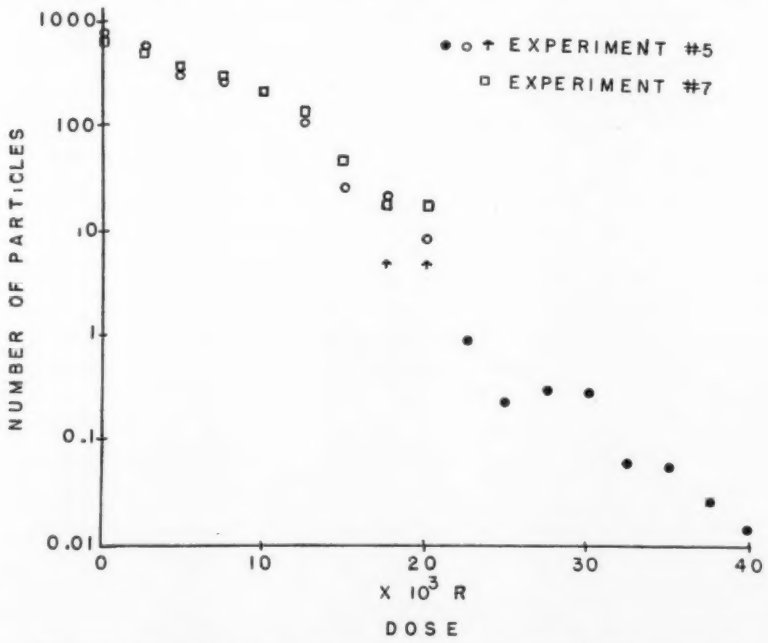


FIGURE 11.—Closed circles and arrows (see p. 359) represent mean numbers of kappa particles plotted logarithmically against X-ray dose. Open circles and squares are means of counts of stainable bodies.

of Equation (1) to calculate the mean particle numbers. The two small arrows are based on zero proportions and represent the lower limit of the best sample estimate of the mean number of particles. They indicate that the best estimate of the points lies above the position of the arrows (See p. 352). The points for number of stainable bodies and number of kappa particles do not conform to the expected straight line; nevertheless, it appears that all points constitute a single inactivation curve, and that kappa particles and stainable bodies are thus identical.

Although the deviation of the whole curve from a straight line may indicate that the inactivation of kappa does not follow a simple exponential one-hit relation as assumed, there are certain other possibilities which might account for the shape of the curve. First, as already pointed out, if any multiplication of viable bodies occurred during the 72 hour interval between radiation and fixation, the points for bodies would tend to be too high, particularly for higher doses of radiation. This would tend to distort the straight line exponential relation into a curve somewhat as observed. Second, failure of all nonviable bodies to disintegrate completely would also give this result. On the other hand, since the initial part of the curve from 0–15,000 r conforms fairly well to a straight line, it may represent a true exponential one-hit curve; and the points of the curve from 15,000 to 40,000 r may be progressively too low due to indirect inactivation effects resulting from the relatively great X-ray dosages. LEA (1947) points out that at high doses typical one-hit exponential inactivation curves often fall off too rapidly due to secondary effects. This is plausible in this case when the extremely great range of the survival curve is considered—extending from approximately 10^3 particles per animal (100 percent survival) down to approximately 10^{-2} particles per animal (0.001 percent survival). If this explanation is correct and the first part of the curve represents a true one-hit exponential survival curve, the inactivation dose may be estimated by inspection from the curve to be about 10,000 r in comparison to 3400–4000 r estimated on the basis of the kappa inactivation data. Which of these explanations is correct cannot be decided on the basis of existing data. It is clear that the simple exponential inactivation of kappa originally postulated must be tested critically by further experiments before it can be accepted.

4. It has already been pointed out that the cytological work discussed in this paper was begun because of the discovery of the great sensitivity of kappa to X-rays, suggesting a size within the range of the ordinary light microscope. Evidence has been presented which indicates that stock G kappa has an inactivation dose in the neighborhood of 3400–4000 r, possibly as high as 10,000 r. Low doses such as this have often been reported for bacteria and other cells, higher doses being characteristic of viruses and enzymes. Only in the case of a few of the larger viruses does the inactivation dose approach this range. In general, an inverse relation is found between size and inactivation dose, the relation being more or less precise and calculable for the smaller viruses. The conclusion reached from a consideration of the inactivation dose is that kappa probably has a size within the range of the bacteria and large viruses. Such a size, of course, agrees well within the observed size of the stainable bodies.

5. VAN WAGTENDONK (1948) has presented evidence that paramecin, the killer substance, is probably a desoxyribonucleoprotein. It seemed likely that kappa, being closely related to paramecin, might also contain DNA. This was one reason for repeated attempts to stain kappa with the Feulgen stain. The fact that the bodies do stain with Feulgen indicates that they probably contain DNA and hence provides additional evidence for the identity of the bodies and kappa.

The DNA content of the bodies is further confirmed by enzyme studies. Crystalline ribonuclease obtained from Worthington Laboratories and desoxyribonuclease generously supplied by DR. MACLYN MCCARTY was used as prescribed by SANDERS (1946). Killers treated with ribonuclease and stained according to the Feulgen technique showed the usual Feulgen positive cytoplasmic bodies. Killers treated with ribonuclease and stained by Giemsa (without acid hydrolysis) showed the bodies just as they did when acid hydrolysis was used instead of the enzyme. Killers treated with ribonuclease, then desoxyribonuclease in the presence of magnesium revealed no cytoplasmic bodies with Feulgen or Giemsa. Specificity of the action of the desoxyribonuclease preparation was indicated by its inability to prevent staining of the bodies in the absence of magnesium. The enzyme results give additional evidence for the desoxyribonucleic acid content of the bodies. The action of ribonuclease is also in agreement with the conclusion (VENDRELY and LIPARDY 1946) that acid hydrolysis acts to break down cytoplasmic ribonucleic acid before desoxyribonucleic acid loses its affinity for the stain. The high concentration of cytoplasmic ribonucleic acid and resulting cytoplasmic basophilia in Paramecium tends to obscure the bodies and probably accounts for the failure of early cytological efforts to differentiate the bodies.

6. The appearance among the bodies of the double forms already referred to suggests that the bodies increase by dividing into two. Such a method of increase agrees with the observations that both the stainable bodies and kappa exist in the form of self-perpetuating particles which increase exponentially, and provides further evidence for the identity of the two.

CONCLUSIONS

In view of the evidence which has been presented, it is concluded that the stainable bodies demonstrated in these experiments actually represent kappa particles. Furthermore, the direct observations recorded here confirm previous inferences made about the properties of kappa on theoretical and experimental grounds, such as localization in cytoplasm, particulate nature, logistic increase, number, size, etc.

One of the most perplexing problems in the study of the killer character in *P. aurelia* has been that of the origin of kappa. For recent detailed discussions of this question, the reader is referred to SONNEBORN (1948, 1949). His major conclusions, summarized briefly, are: (1) Since X-ray data and staining show a large size for kappa, it is unlikely that it is a gene derivative, as once suggested. (2) There is no decisive evidence bearing on the question of whether kappa is internal or external in origin. (3) There is no fundamental difference

between intracellular symbionts as small and simply organized as kappa and genetic elements localized in the cytoplasm. Kappa appears to satisfy the requirements for both—there being no distinction practical in the case of such bodies. Thus while it is possible that kappa is a recent intruder, evolutionarily speaking, it is also possible that it arose from some cellular element present in Paramecium. Since only killer strains of *P. aurelia* contain stainable cytoplasmic bodies, it is unlikely that kappa represents one of a group of essentially similar cytoplasmic factors. Since it is unlikely that kappa is a gene derivative, kappa probably has no direct relation to theories of gene action which hypothesize gene-initiated genetic cytoplasmic particles.

The question of whether kappa resembles any known cytoplasmic elements deserves some comment. In regard to the suggestion (LINDEGREN 1946) that kappa is a symbiotic virus, it should be noted that although the large viruses are as large as the smallest kappa particles, most kappa particles are considerably larger than any known virus (cf. ALTENBURG 1948). Relative to ALTENBURG'S suggestion that kappa represents an algal derivative (ALTENBURG 1946, 1948), the structure and size of kappa would seem to set it apart sharply from any known algae. Acid hydrolysis and Giemsa staining of *P. bursaria* reveals the zoochlorellae as large clearly stained cells three to four micra in diameter, with a spherical nucleus about 1.5 micra in diameter. The cell wall is plainly visible.

Perhaps the known DNA containing cytoplasmic elements which, at least superficially, most resemble kappa are certain of the intracellular symbionts such as the Rickettsiae. Both are of comparable size and general appearance and are restricted to an intracellular habitat. Rickettsiae are apparently nonpathogenic in their arthropod hosts. However, recent work on the cytology of *Rickettsia prowazeki* (RIS and FOX 1949) has shown that the bodies are differentiated into chromatinic ("nuclear") portions containing DNA and a "cytoplasmic" portion containing RNA. Of the various methods used to demonstrate this, one was the technique used here for kappa—Giemsa preceded by acid hydrolysis. No evidence for any "cytoplasmic" region has been observed in our preparations although particular attention has been given to this point. However, this observation cannot be considered decisive, for the conditions under which kappa is observed, i.e. surrounded by cytoplasm of the Paramecium, would probably tend to obscure any lighter staining areas about the DNA containing bodies. Nevertheless, caution should certainly be observed in comparing kappa with such symbionts as Rickettsiae and bacteria until the structure of kappa is better known. As far as we know now, kappa appears to resemble the "nuclear" apparatus of Rickettsiae or bacteria more than it does the whole of those organisms.

Whatever the structure and origin of kappa, it now represents a very integral part of Paramecium, conferring immunity to the poison produced as a result of its presence, dependent on a specific dominant gene, competing with another cytoplasmic element (sigma), and perhaps giving the Paramecium a selective advantage in nature—appearing in these roles as an hereditary determiner localized in the cytoplasm, yet at the same time having the qualities of an obligatory symbiont.

SUMMARY

Both the Feulgen reaction and acid hydrolysis followed by Giemsa reveal microscopic bodies in the cytoplasm of killers of *Paramecium aurelia*. The bodies are 0.2 to 0.8 micron in size, variable in form, appearing often as rods or spheres. They range from 400–1200 in strong killers of stock G. They contain DNA. Evidence is presented which indicates that these bodies are identical with the cytoplasmic factor kappa. The significance of these findings is discussed.

ACKNOWLEDGEMENTS

The author is indebted to MISS ROSEMARY GILMARTIN for technical assistance.

LITERATURE CITED

ALTENBURG, E., 1946 The symbiont theory in explanation of the apparent cytoplasmic inheritance in Paramecium. Amer. Nat. **80:** 661–662.

1948 The role of symbionts and autocatalysts in the genetics of the ciliates. Amer. Nat. **82:** 252–264.

LEA, D. E., 1947 Actions of radiations on living cells. xii+402 pp. Cambridge: At the University Press, New York: The Macmillan Company.

LINDEGREN, C. C., 1946 See "Discussion" in Sonneborn, T. M., 1946 Experimental control of the concentration of cytoplasmic genetic factors in Paramecium. Cold Spring Harbor Symp. Quant. Biol. **11:** 236–255.

PREER, J. R., 1946 Some properties of a genetic cytoplasmic factor in Paramecium. Proc. nat. Acad. Sci. **32:** 247–253.

1948a A study of some properties of the cytoplasmic factor, "kappa," in *Paramecium aurelia*, variety 2. Genetics **33:** 349–404

1948b The killer cytoplasmic factor kappa: its rate of reproduction, the number of particles per cell, and its size. Amer. Nat. **82:** 35–42.

1948c Microscopic bodies in the cytoplasm of "killers" of *Paramecium aurelia* and evidence for the identification of these bodies with the cytoplasmic factor, kappa. Genetics **33:** 625.

RAFALCO, J. S., 1946 A modified Feulgen technic for small and diffuse chromatin elements. Stain Tech. **21:** 91–93.

RIS, H., and J. P. FOX, 1949 The cytology of rickettsiae. J. exp. Med. **89:** 681–686.

SANDERS, F. K., 1946 Cytochemical differentiation between the pentose and desoxypentose nucleic acids in tissue sections. Quart. J. micr. Sci. **87:** 203–208.

SONNEBORN, T. M., 1943 Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *Paramecium aurelia*. II. The bearing of the determination and inheritance of characters in *Paramecium aurelia* on the problems of cytoplasmic inheritance, pneumococcus transformations, mutations and development. Proc. nat. Acad. Sci. **29:** 329–343.

1948 Symposium on plasmagenes, genes, and characters in *Paramecium aurelia*. Introduction. Amer. Nat. **82:** 26–34.

1949 Beyond the gene. Amer. Scient. **37:** 33–59.

SONNEBORN, T. M., and R. V. DIPPELL, 1946 Mating reactions and conjugation between varieties of *Paramecium aurelia* in relation to conceptions of mating type and variety. Physiol. Zool. **19:** 1–18.

VAN WAGTENDONK, W. J., 1948 The action of enzymes on paramecin. J. biol. Chem. **173:** 691–704.

VENDRELY, R., and J. LIPARDY, 1946 Acides nucléiques et noyaux bactériens. C. R. Acad. Sci., Paris **223:** 342–344.

WOLBACH, S. B., 1919 Studies on Rocky Mountain spotted fever. J. med. Res. **41:** 1–197.

INHERITANCE OF FRUIT WEIGHT AND EARLINESS IN A TOMATO CROSS[1]

H. W. FOGLE AND T. M. CURRENCE
University of Minnesota, St. Paul, Minnesota

Received November 19, 1949

ACKNOWLEDGE of the inheritance of certain complex characters such as fruit weight and earliness may be applied directly to a tomato breeding program. LINDSTROM (1924), MACARTHUR (1934a), YEAGER (1937), CURRENCE (1938) and others have reported studies on associations between known qualitative characters and certain quantitative characters. A method of estimating the number of gene pairs differentiating parent plants for a quantitative character and the nature of dominance and interactions of these genes by detailed analysis of segregating and non-segregating generations has been suggested by POWERS, LOCKE, and GARRETT (1949)

MATERIALS AND METHODS

The present study was attempted to obtain further information on earliness and fruit size. An analysis was made of parental, F_1, F_2, and backcross generations from a cross of Tiny Tim by Stemless Pennorange. Tiny Tim, a dwarf, small-fruited variety introduced by the NEW HAMPSHIRE EXPERIMENT STATION, was derived from a cross of Window Box, a dwarf ornamental, and *Lycopersicon pimpinellifolium*. Individual plant selections had been made in two seasons prior to this study and the resulting plants appeared uniform. Pennorange is a "stemless" variety (jointless pedicel) having also the other recessives tangerine-orange flesh color, colorless skin, wiltiness of foliage, and uniform coloring of fruit. This variety was derived from a cross of Marglobe and a French "stemless" variety, Rouge Naine Hative, by PENNSYLVANIA STATE COLLEGE. A seed sample of Pennorange was obtained in 1944 and individual plant selections were made in three seasons previous to using it as a parent.

Crosses were made between individual plants of the parents. Cuttings of the original parent plants and a single F_1 plant were used in producing backcross generations and the F_2. Selfed seed from the parent plants were used as the parental progenies. The statistical design of the field planting was a randomized complete block consisting of nine replications. Seventeen plots of ten plants each comprised a replication: one plot each of the parents and F_1, three plots of each backcross, and eight plots of F_2. Spacing was six by six feet, which was thought sufficient to eliminate competition between plants. All data were taken on an individual plant basis. Components of fruit weight and earliness were analyzed by two methods: (1) the partitioning method and (2) association with qualitative characters used as chromosomal markers.

[1] Paper No. 2535 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

EXPERIMENTAL RESULTS

Segregation of Qualitative Characters

Each character thought to be simply inherited was tested for agreement to a monohybrid ratio. Flesh color, skin color, wiltiness of foliage, and uniform color of unripe fruit gave good agreement to single factor ratios. There was a deficiency of plants in the recessive class for plant type and pedicel type in the F_2 but dominant and recessive classes were approximately equal in the backcrosses. It is quite possible therefore that the deficiency of recessives was due to lower viability of the recessive type or a similar cause. The simply inherited characters were easily classified due to distinct segregation, so it was thought that errors in classification were not of importance. Independence was tested by Chi-square on various combinations of the characters in the F_2 and backcrosses. Poor agreement to a dihybrid ratio was obtained when flesh color and uniformity were combined, with recombination values of 37 ± 2.4 percent in F_2 and 41 ± 3.0 percent in the backcross to Pennorange, suggesting that the flesh color gene was *t*, which has been reported by MACARTHUR (1934b) on chromosome seven at 40 map units from *u*. Pedicel type and wiltiness of foliage showed recombination values of 30 ± 3.6 in the backcross to Pennorange and 32 ± 2.2 percent in the F_2. MACARTHUR (1934b) placed *wt* on chromosome ten but the above linkage with pedicel type seems fairly definite and for purposes of this paper both jointless pedicel and wilty foliage are considered to be on chromosome five. MACARTHUR'S test did not include the jointless pedicel gene but close linkage of pedicel type with three gene pairs on chromosome five was reported by BUTLER (1936). Linkage was not suggested by combinations of other characters. It was concluded therefore that chromosome one was marked by the dwarfness character, three by skin color, five by pedicel type and wiltiness of foliage and seven by flesh color and uniform color of unripe fruit.

Locule Number

Locule number is noted in the literature as being affected by relatively few genes and it is readily recorded in quantitative classes. It is therefore an interesting component of fruit size when studied by the partitioning-of-genotypes method and by association with qualitative characters.

The number of segregating gene pairs and the nature of interactions may be estimated as suggested by POWERS, LOCKE, and GARRETT (1949). Means and total variances are used in estimating genetic variances for each generation to determine the degree of homozygosity of the parents. From a study of dominance and a frequency distribution of the individual data expressed as percentages, an hypothesis is formulated as to the number of gene pairs segregating and as to the genotypes of the parents. The hypothesis may then be tested by a detailed partitioning of the segregating generations into possible genotypes. SINGH (1949) summarizes the application of the partitioning method in an inheritance study of a *Cucurbita maxima* cross.

POWERS (1939), MACARTHUR (1941) and others have reported that locule

number inheritance approaches a geometric progression. In such data logarithms of the individual data are normally distributed and conversion of the data is necessary to obtain an accurate estimate of total variance. Logarithms of the parental and F_1 means gave better predictions of the segregating generation means than did the original means. Geometric inheritance of locule number therefore was assumed.

Environmental variances in table 1 were predicted from the regression of

TABLE 1

Individual plant means, variances calculated from logarithms, and condensed frequency distributions for number of locules per fruit

GENERATION	NUMBER OF PLANTS	LOCULE NO. (LOGARITHM OF MEAN)	VARIANCES			FREQUENCY DISTRIBUTION		
			TOTAL	ENVIRON.	GENETIC	2–4	5–6	7–14
Tiny Tim	90	0.510	0.0033	0.0032	0.0001	100.0	0.0	0.0
BC to P-1	269	0.550	0.0025	0.0038	−0.0013	94.8	5.2	0.0
F_1	90	0.600	0.0058	0.0046	0.0012	80.1	19.9	0.0
F_2	718	0.645	0.0118	0.0053	0.0065	58.8	37.3	3.9
BC to P-2	267	0.715	0.0144	0.0064	0.0080	34.8	48.0	17.2
Pennorange	90	0.932	0.0085	0.0099	−0.0014	0.0	13.3	86.7

total variances on means in the non-segregating generations, consisting of the parents and F_1. Genetic variances were obtained by subtracting environmental from total variance. Since parental and F_1 genetic variances were small in relation to that of the F_2 and of the backcross to the parent tending to be recessive, the parents were relatively homozygous for genes affecting locule number.

A frequency distribution with locule number classes from two to fourteen was converted to percentages and grouped into classes of 2–4, 5–6, and 7–14 locules per fruit to give maximum differentiation of the parents and F_1. The term penetrance is used hereafter to denote the percentage of a population in a given class. The approximate number of segregating gene pairs was estimated to be between two and three, since the F_2 penetrance was 4.5 percent and the backcross to Pennorange 19.8 percent that of Pennorange in the 7–14 locule class. Dominance studies showed at least one of the factor pairs to have a greater effect on locule number than the others and fewer locule number approached a single factor difference, suggesting an hypothesis of a major factor and two minor factors of equal effect segregating in this cross. When penetrances and theoretical percentages for genotypes in the F_2 and backcrosses were estimated by this hypothesis, good agreement of calculated to observed numbers was found. In the F_2, calculated numbers were 447, 244 and 27 and observed 422, 268, and 28 respectively in the three classes. In the backcrosses, Chi-square values with probabilities in excess of 0.95 were ob-

tained. Each segregating generation therefore supports the hypothesis that locule number in this cross was controlled by a major and two minor genes.

Frequency distributions of locule number in dominant and recessive classes of each of the qualitative characters marking four chromosome pairs were compared and differences were tested for significance by the *t* test. Comparisons made in the F_2 and the backcross to the recessive parent for each qualitative pair are shown in table 2. Differences between dominant and recessive classes are given in the final column.

TABLE 2

Mean locule number of plants in populations segregating for genes used to mark four chromosome pairs

GENOTYPE OR PHENOTYPE	CHROMOSOME PAIR	GENERATION	NO. OF PLANTS	NUMBER OF LOCULES		
				DOMINANT	RECESSIVE	DIFFERENCE
ddYYJJTTUUW^{t}W^t		Tiny Tim (P-1)	90	3.24	—	—
DDyyjjttuuw^{t}w^t		Pennorange (P-2)	90	8.51	—	—
DdYyJjTtUuW^{t}w^t		F_1	90	3.98	—	—
Standard: dwarf	1	BC to P-1	269	3.64	3.43	0.21**
D : *d*		F_2	718	4.54	3.95	0.59**
Yellow: colorless	3	BC to P-2	267	5.00	5.43	−0.43*
Y : *y*		F_2	718	4.39	4.51	−0.12
Jointed: jointless	5	BC to P-2	267	5.27	5.11	0.16
J : *j*		F_2	718	4.47	4.27	0.20
Normal: wilty leaf	5	BC to P-2	267	5.33	5.07	0.26
W^t : *w^t*		F_2	718	4.47	4.33	0.14
Red flesh: orange	7	BC to P-2	267	5.01	5.44	−0.43*
T : *t*		F_2	718	4.35	4.65	−0.30**
Green base: uniform	7	BC to P-2	267	5.11	5.31	−0.20
U : *u*		F_2	718	4.44	4.37	0.07

* Sig. diff. (5%).
** Sig. diff. (1%).

A highly significant difference between dwarf and standard type plants was noted. Fewer locule number of *dd* plants in both the backcross to Tiny Tim and the F_2 suggest that a dominant gene (or more than one) for locule number was brought into the cross by the *dd* parent. On chromosome seven a significant association between red flesh and fewer locule number in the backcross and a highly significant one in the F_2 were noted. No significant association between

dark green base on the fruit and fewer locule number was observed in either segregating generation. Since *T* and *U* are linked with approximately 40 percent recombination, a factor for locule number nearer to the *T* locus than to *U* on chromosome seven is suggested. The dominant of this locule-number gene apparently was introduced into the cross by Tiny Tim in coupling with *T* and U. There is also a suggestion of a third factor pair for locule number on chromosome three, with low locule number brought into the cross by Tiny Tim in coupling with the gene *Y*. A significant association between yellow skin and fewer locule number was noted in the backcross population but in the F_2 the difference was not significant although in the same direction. No association of locule number with qualitative characters marking chromosome five was noted.

Since genic effects are multiplicative in data showing geometric inheritance, interactions between gene pairs might be expected. Locule number of F_2 phenotypes of the unit characters found associated with locule number were compared in an attempt to detect such interactions. Table 3 shows the means and differences. Differences in locule number between standard and dwarf plants were compared when flesh color and skin color were both recessive, when one was dominant and the other recessive, and when both were dominant. The difference of 0.23 ± 0.53 between *Dty* and *dty* is not significant but the other three differences, 0.92 ± 0.25, 0.72 ± 0.26, and 0.45 ± 0.13 are highly significant. In no comparison is the difference between differences significant but with the small number of plants in the *dty* group and the resulting high standard error for the group the possibility of gene interaction cannot be eliminated. It seems apparent that the dominant associated with *d* may not be as effective in reducing number of locules when present with *t* and *y* as it is with *T*, *Y*, or *TY*. Similar comparisons made for flesh color and skin color show a difference of 0.47 ± 0.12 between *DYt* and *DYT* and one of 0.25 ± 0.11 between *DTy* and *DTY*. Other differences were not significant which suggests that the genes for locule number in these two chromosomes also may be more effective in some combinations than they are in others.

The phenotypic class *dTY* had a slightly higher locule number than the dominant parent, Tiny Tim. Apparently one or more genes affecting locule number were present in the cross in chromosomes other than those carrying the genes for qualitative characters. Such a gene could of course be in the same groups but located too distant from the *d*, *y*, and *t* genes to show association with them. Mention should also be made of the fact that the data presented on the quantitative characters do not eliminate the possibility that the genes for the qualitative characters may themselves affect number of locules rather than genes linked with them.

Weight per Locule

Size of locules or weight per locule was shown by POWERS, LOCKE, and GARRETT (1949) also to be a component of fruit weight. In the present study, individual plant data were studied to determine whether geometric processes were involved as in locule-number inheritance. In each segregating generation,

TABLE 3

Locule number of various F_2 phenotypes of three qualitative characters.

PHENOTYPE	NO.	LOCULE NO.	DIFF.
(a) *Dd* comparisons—locule no. dominant assoc. with *d*			
Dyt	32	4.56±0.21	0.23±0.53
dyt	6	4.33±0.49	
DYt	107	4.84±0.10	0.92±0.25
dYt	27	3.92±0.23	
DyT	139	4.62±0.09	0.72±0.26
dyT	30	3.90±0.24	
DYT	299	4.37±0.07	0.45±0.13
dYT	77	3.92±0.11	
(b) *Tt* comparisons—locule no. dominant assoc. with *T*			
Dyt	32	4.56±0.21	0.06±0.23
DyT	139	4.62±0.09	
DYt	107	4.84±0.10	0.47±0.12
DYT	299	4.37±0.07	
dyt	6	4.33±0.49	0.43±0.54
dyT	30	3.90±0.24	
dYt	27	3.92±0.23	0.00±0.25
dYT	77	3.92±0.11	
(c) *Yy* comparisons—locule no. dominant assoc. with *Y*			
Dty	32	4.56±0.21	0.28±0.23
DtY	107	4.84±0.10	
DTy	139	4.62±0.09	0.25±0.11
DTY	299	4.37±0.07	
dty	6	4.33±0.49	0.41±0.52
dtY	27	3.92±0.23	
dTy	30	3.90±0.24	0.02±0.26
dTY	77	3.92±0.11	

observed locule weights were definitely lower than predicted if arithmetic processes were assumed but not significantly different from predicted means assuming geometric inheritance. Means in terms of logarithms were predicted to be 0.444, 0.726, and 1.009 in the backcross to Tiny Tim, F_2 and backcross to Pennorange, respectively, which agree closely with logarithms of the observed means for these generations given in the third column of table 4. Individual plant data converted to logarithms therefore were used in calculating total variances. Table 4 presents logarithms of means, total variances, and

calculated values for environmental and genetic variances. Genetic variances for the parental and F_1 generations were small in relation to the segregating generations suggesting that both parents were homozygous for weight per locule. Dominance appeared to be lacking since the F_1 was intermediate and the F_1 and F_2 were not significantly different in locule size.

Three classes, having approximately equal intervals in terms of logarithms, gave maximum differentiation of the parental and F_1 generations: 0.1 to 2.0, 2.1 to 16.5 and 16.6 or more grams. The last three columns of table 4 are the

TABLE 4

Individual plant means, variances calculated from logarithms, and condensed frequency distributions for weight per locule of fruit.

GENERATION	NUMBER OF PLANTS	LOCULE WT. (LOGARITHM OF MEAN)	VARIANCES			FREQUENCY DISTRIBUTION		
			TOTAL	ENVIRON.	GENETIC	0.1–2.0	2.1–16.5	16.6+
Tiny Tim	90	0.149	0.0043	0.0024	0.0019	100.0	0.0	0.0
BC to P-1	268	0.462	0.0152	0.0070	0.0082	13.8	86.2	0.0
F_1	90	0.739	0.0068	0.0103	−0.0035	0.0	100.0	0.0
F_2	718	0.736	0.0320	0.0103	0.0217	2.1	97.8	0.1
BC to P-2	266	1.003	0.0322	0.0138	0.0184	0.4*	92.2	7.4
Pennorange	90	1.279	0.0191	0.0175	0.0016	0.0	34.4	65.6

* 1 plant, partially sterile.

condensed distribution into these classes. Class three appears to consist entirely of individuals of the Pennorange genotype and direct comparisons of generations can be made in this class. In the F_2, 1.56 percent of the plants, and in the backcross to Pennorange 11.28 percent, are of the Pennorange genotype. With three factors segregating, 1.56 and 12.5 percent respectively are expected. Tiny Tim appears homozygous for factors producing smaller locule weight and Pennorange for factors producing greater weight. A penetrance of 0.4 into class one by the backcross to Pennorange was caused by a single plant which showed a tendency to form seedless fruit. Thus three factors, lacking dominance, for locule weight are assumed. When this hypothesis was applied to the backcrosses and F_2, good agreement of predicted to observed means was obtained. In the F_2, predicted numbers were 15,696 and 7 in classes one, two, and three and observed numbers were 15,702, and 1. In the backcross to Tiny Tim, calculated and observed numbers were identical and in the backcross to Pennorange predicted numbers were 0,244, and 21 and the observed, 1,246, and 19.

Table 5 summarizes associations found between locule weight and qualitative characters used to mark four chromosome pairs. Significant associations were noted on chromosomes three, five, and seven. In each instance, smaller locule weight was found associated with dominant genes from Tiny Tim. On

chromosome seven, a locus for locule weight gave a significant decrease in the *U* class but only a small reduction in the *T* class, while the opposite appeared true with the gene for locule number on chromosome seven. It is possible then that two genes affecting size of fruit are present on chromosome seven.

TABLE 5

Mean weight per locule of plants in populations segregating for genes used to mark four chromosome pairs.

GENOTYPE OR PHENOTYPE	CHROMOSOME PAIR	GENERATION	NO. OF PLANTS	LOCULE WEIGHT IN GRAMS		
				DOMINANT	RECESSIVE	DIFFERENCE
ddYYJJTTUUW^{t}W^t		Tiny Tim (P-1)	90	1.19	—	—
DDyyjjttuuw^{t}w^t		Pennorange (P-2)	90	19.10	—	—
DdYyJjTtUuW^{t}w^t		F_1	90	3.04	—	—
Standard: dwarf	1	BC to P-1	268	2.98	2.81	0.17
D : d		F_2	718	5.47	5.09	0.38
Yellow: colorless	3	BC to P-2	266	9.53	10.25	−0.72
Y : y		F_2	718	5.07	6.19	−1.12**
Jointed jointless	5	BC to P-2	266	9.26	10.51	−1.25*
J : j		F_2	718	5.38	5.40	−1.02
Normal: wilty leaf	5	BC to P-2	266	8.98	10.81	−1.83**
W^t : w^t		F_2	718	5.24	5.87	−0.63**
Red flesh: orange	7	BC to P-2	266	10.26	10.95	−0.69
T : t		F_2	718	5.35	5.48	−0.13
Green base: uniform	7	BC to P-2	266	8.47	11.12	−2.65**
U : u		F_2	718	5.23	5.73	−0.50

* Sig. diff. (5%).
** Sig. diff. (1%).

Differences in locule weight between distributions of dominant and recessive classes of *Yy*, *Uu*, and *Wtwt* in the backcross to Pennorange were compared for each locus when the other two loci were dominant or recessive. In each case highly significant differences were found between means of the dominant and recessive classes for one of the loci if the other two loci had genes associated with smaller locule weight but were not different if the other loci had genes associated with greater locule weight. The difference between *WtUY* and *wtUY*, for example, was 3.78 ± 0.86 grams while that between *Wtuy* and *wtuy* was 0.04 ± 0.87. The difference between these differences rather definitely suggests gene interactions. The *Yy* locus showed a significant difference in

like comparisons and the *Uu* locus gave a difference in the same direction and approaching significance. In the Pennorange backcross *wtuy* had a mean of 10.9 ± 0.68 while Pennorange, which is of the same phenotype, had a mean of 19.1 ± 0.63. Additional genes seem a possible reason for the difference.

By the partitioning analysis, three major gene pairs, lacking dominance, appeared to be segregating for weight per locule but the sizable difference between Pennorange and the *wtwtuuyy* backcross group suggests the possibility of other important genes for the character. Geometric inheritance of locule size was found. Significant interactions between two gene pairs in comparisons and a trend in the same direction for the third also suggests geometric processes. Genes affecting number of locules were found for chromosomes one, three, and seven and for weight of locules, they were in three, five and seven. In all then, four groups affect fruit size with a suggestion that genes for the two components are distinct in chromosome seven.

Fruit Weight

Fruit weight inheritance was studied in order to compare results with those obtained when the two components, locule number and weight per locule, were analyzed. Geometric action of the genes was noted the same as for both components.

Three fruit-weight classes gave maximum differentiation of parental and F_1 populations. These classes, 0–10, 10.1–100, and 100.1–235 grams, were used in a condensed frequency distribution with class 3 consisting apparently of the Pennorange genotype. Direct comparisons of generations with penetrance into class three therefore were possible. F_2 penetrance in class three was 0.11 percent and the backcross to Pennorange 4.18 percent that of Pennorange. Assuming five factors, 0.10 percent in F_2 and 3.12 percent in the backcross would be expected to have the Pennorange genotype. With five segregating gene pairs, detailed analysis of genotypes is not feasible because estimating penetrances for a large percentage of the genotypes becomes complex and inaccurate. The number of genes postulated thus for fruit weight is a reasonable approximation to the six found for the two components.

Associations were found between fruit weight and qualitative characters marking four pairs of chromosomes. Highly significant associations of smaller fruit weight were found with *d* on chromosome one, with yellow skin on chromosome three, and with red flesh and dark green base on chromosome seven. A significant association of smaller weight with *W'* on chromosome five was found in the F_2 and in the same direction and approaching significance in the backcross. In each instance, smaller fruit weight was associated with qualitative genes contributed to the cross by Tiny Tim. Four chromosome pairs therefore have one or more gene pairs segregating for fruit weight in this cross. Factors for both components have been noted on chromosome seven. A locule-number locus was indicated closely associated with the *Tl* locus and a locule-weight one with the *Uu* locus. Factors for both number and weight of locules were found on chromosome three, also, but whether these were due to a single locus could not be determined because a single marker gene pair was used.

Associations noted between fruit weight and the qualitative gene characters appeared to be on the same chromosomes that had the genes for locule number and locule weight.

Number of Days from Seeding to Flowering

Division of the time from seeding to fruit ripening into smaller component stages may simplify an inheritance study of earliness. POWERS and LYON (1941) suggest three such components: number of days from seeding to first blossom, first blossom to first fruit set, and first fruit set to first ripe fruit. Further subdivision would be desirable but biological divisions for other stages are not apparent. In the present study, earliness and the three component periods were recorded for individual plants.

It will be noted in table 6 that Tiny Tim had nineteen days shorter duration of the first period than Pennorange and the F_1 mean, 55.17 ± 0.41 was significantly lower than the predicted, 59.01 ± 0.47. Predicted values for the segregating generations were not different from observed means assuming arithmetic inheritance. Geometric processes therefore were not evident for this period and total variances were calculated from actual number of days for individual plants. Genetic and environmental variances were not estimated since total variances suggested that the expected straight line relationship between generation means and environmental variances was not present. The Tiny Tim parent may have been heterozygous for genes controlling this stage or environment may have affected the relationship. This stage included transplanting to the field and initiation of flowering, so that differential response to various environmental factors may have occurred. The later generations, Pennorange, the F_2, and the backcross to Pennorange, were probably brought into blossom more uniformly than earlier generations as a result of warmer weather toward the end of the period. This would reduce variances of these generations having larger means. Tiny Tim seemed to be relatively homozygous for earliness factors in the second and third stages as well as for the entire period and for all qualitative characters studied. It might be expected therefore to be homozygous also for the first stage and that environ-

TABLE 6

Individual plant means, total variances, and condensed frequency distribution for number of days from seeding to first blossom.

GENERATION	NUMBER OF PLANTS	MEAN NUMBER DAYS	TOTAL VARIANCE	FREQUENCY DISTRIBUTION		
				43–57	58–67	68–87
Tiny Tim	90	49.58 ± 0.38	11.62	97.8	2.2	0.0
BC to P-1	270	51.78 ± 0.28	14.36	89.7	10.3	0.0
F_1	90	55.17 ± 0.41	17.22	70.0	30.0	0.0
F_2	720	59.92 ± 0.16	18.01	24.4	73.0	2.6
BC to P-2	270	62.97 ± 0.25	15.07	7.4	80.0	12.6
Pennorange	89	68.45 ± 0.28	3.82	0.0	16.7	83.3

mental influence caused a large variance for the period from seeding to flowering.

Maximum differentiation of the parental and F_1 generations was obtained by condensing the frequency distribution into three classes: 43–57, 58–67, and 68–87 days as shown in table 6. Class three apparently contains only those genotypes as late as or later than Pennorange. Since 3.12 percent of the F_2 and 15.12 percent of the backcross to Pennorange are similar in genotype to Pennorange, an estimate of three factor pairs differentiating the parents with dominants at each locus from Tiny Tim appears valid.

A three factor hypothesis gives a combined F_1 and Pennorange penetrance in class one of 8.75 percent whereas the total backcross to Pennorange penetrance in class one is 7.4. This suggests that more than three genes may be involved. In the F_2, observed penetrance in the 43–57 day class was significantly lower than predicted by a three-factor hypothesis and penetrances were also lower than predicted in the backcrosses. If four or more gene pairs are assumed with one dominant earliness factor from Pennorange and the others from Tiny Tim, class three penetrances are not changed appreciably but calculated penetrances in class one are lowered. An hypothesis of four gene pairs gave better agreement with the observed segregating generation penetrances but still differed by borderline significance. More than four gene pairs therefore appear possible since additional ones would tend to give better agreement. Due to the large number of genotypes with unknown penetrances which must be estimated, partitioning of genotypes becomes too complex for ready solution.

In table 7 associations between the first stage of earliness and qualitative markers on different chromosome pairs are summarized. Significant associations of shorter duration with dominant markers on each of chromosomes one, three, five, and seven were noted. On chromosome one, earliness was associated with standard plant type of Pennorange, but other associations were with dominants from Tiny Tim. At least four gene pairs therefore would be estimated to differentiate the parents for duration of the first period. Eight chromosome pairs were not marked and associations were possible on any of these. Interallelic interactions were not evident in comparisons of dominant and recessive classes of each marker gene pair showing assocation with earliness when the other marker genes were all dominant or all recessive. A slightly greater increase in earliness due to the presence of a dominant marker was noted in each instance when the other markers were also dominant, but the differences did not approach significance. Effects of the gene pairs in reducing the duration of the period therefore appeared to be approximately additive.

The two types of analyses suggest four or more gene pairs affecting the period from seeding to flowering in this cross. There was a suggestion by the partitioning method that three of the gene pairs have relatively greater effect than the others, but this was not apparent in the association tests. For each of the four gene pairs affecting this period, earliness occurred in coupling with the genes used for chromosomal markers. One dominant for shorter duration apparently came from Pennorange and the other three from Tiny Tim. It is

TABLE 7

Mean number of days from seeding to first blossom of plants in populations segregating for genes used to mark four chromosome pairs.

GENOTYPE OR PHENOTYPE	CHROMOSOME PAIR	GENERATION	NO. OF PLANTS	NUMBER OF DAYS		
				DOMINANT	RECESSIVE	DIFFERENCE
ddYYJJTTUUW^{t}W^t		Tiny Tim (P-1)	90	49.38	—	—
DDyyjjttuuw^{t}w^t		Pennorange (P-2)	89	69.44	—	—
DdYyJjTtUuW^{t}w^t		F_1	90	55.22	—	—
Standard: dwarf	1	BC to P-1	270	51.07	52.92	−1.85**
D : d		F_2	720	59.81	61.36	−1.55**
Yellow: colorless	3	BC to P-2	270	62.24	64.22	−1.98**
Y : y		F_2	720	59.63	61.36	−1.73**
Jointed: jointless	5	BC to P-2	270	61.81	64.44	−2.63**
J : j		F_2	720	59.66	62.42	−2.76**
Normal: wilty leaf	5	BC to P-2	270	62.31	63.96	−1.65**
W^t : w^t		F_2	720	59.78	61.09	−1.31**
Red flesh: orange	7	BC to P-2	270	62.22	63.18	−0.96*
T : t		F_2	720	60.05	61.23	−1.18*
Green base: uniform	7	BC to P-2	270	61.97	63.19	−1.22*
U : u		F_2	720	59.82	61.01	−1.19*

* Sig. diff. (5%).
** Sig. diff. (1%).

possible that the recessive marker genes may delay flowering but no conclusive evidence is available on this possibility in the present study. Shorter duration of the period showed partial dominance over longer duration.

Number of Days from Flowering to Fruit Setting

The period from time of blossoming to fruit setting covers a relatively shorter time than the other two stages of earliness with the parental difference being five and one-half days, which was greater than the mean of the Pennorange parent. Fruit was considered set when the ovary had swollen to at least three times normal unpollinated size and the pedicel had enlarged noticeably. Climatic environment apparently has considerable effect on the duration of this stage since a number of flowers may abscis before fruit sets on a plant. An ability to set fruit under unfavorable conditions such as hot, dry weather might be heritable since Pennorange showed a tendency to fruit more readily than Tiny Tim. Association of this ability with an abscission layer at the pedicel joint in Tiny Tim when fruit fails to set quickly is a possibility but the

jointed F_1 was intermediate and the segregating generations formed distributions that suggested an hereditary basis for this stage. Due to differences in duration of the first stage, earlier plants were not subjected to the same extent as later ones to hot, dry weather but differences seemed definite nevertheless.

Since differences between block means were small, total mean squares were used as the estimates of total variance given in table 8. When esti-

TABLE 8

Individual plant means, variances, and condensed frequency distributions for number of days from first blossom to first fruit set.

GENERATION	NO. OF PLANTS	MEAN NUMBER DAYS	VARIANCES			FREQ. DISTRIBUTION		
			TOTAL	ENVIRON.	GENETIC	3	4–12	13+
Tiny Tim	90	9.83±0.30	8.78	7.16	1.62	0.0	89.0	11.0
BC to P-1	267	8.87±0.13	10.02	6.26	3.76	0.8	95.3	3.9
F_2	718	8.36±0.14	19.59	5.79	13.80	2.6	83.7	13.7
F_1	90	6.92±0.16	1.48	4.46	−2.98	0.0	98.9	1.1
BC to P-2	264	6.91±0.19	11.18	4.45	6.73	4.5	88.6	6.9
Pennorange	89	4.26±0.17	3.34	1.99	1.35	39.3	60.7	0.0

mated environmental variances are removed, the segregating generations show more genetic variance than either parent so it is thought that the parents were relatively homozygous. The F_1 mean of 6.92 ± 0.16 days is not significantly different from a predicted value of 7.04 ± 0.26, suggesting that phenotypic dominance was lacking. However, the F_1 had 1.44 days shorter duration than the F_2, the backcross to Pennorange was significantly higher than a predicted value of 5.59 ± 0.23 assuming no dominance, and the backcross to Tiny Tim was slightly higher than predicted without dominance. A slight dominance of longer duration may be present.

A frequency distribution expressed in percentages is given in the last three columns of table 8. Both Tiny Tim and the F_1 have no penetrance in the three day class and penetrances of the segregating generations therefore require genotypes similar to Pennorange. The F_2 penetrance is 6.61 and the backcross to Pennorange is 11.5 percent that of Pennorange in this class, suggesting two and three factors for shorter duration, respectively, from the Pennorange parent. Penetrance of the backcross to Tiny Tim into this class suggests a factor for shorter duration carried by Tiny Tim but masked by three factors for longer duration. Segregates with both longer and shorter duration than the parents were observed in distributions of the backcross to Pennorange and the F_2. Thus three dominants for shorter duration from Pennorange and one from Tiny Tim are assumed to control this period. Analysis of the segregating generation genotypes suggests complementary action of the three Pennorange factors. The Tiny Tim factor appears to have a greater effect than each of the Pennorange factors. The single genotype *AaBbCcDD*, if *D* represents the Tiny Tim gene for shorter duration, appears to account for the three-day class pene-

trance of the backcross to Tiny Tim. Using this hypothesis there is good agreement between observed penetrances and theoretical percentages.

Table 9 summarizes associations of the second stage with qualitative genes on the four chromosome pairs. Significant associations in F_2 were found on chromosomes three and five and an association approaching significance on

TABLE 9

Mean number of days from first blossom to first fruit set of plants in populations segregating for genes used to mark four chromsome pairs.

GENOTYPE OR PHENOTYPE	CHROMOSOME PAIR	GENERATION	NO. OF PLANTS	NUMBER OF DAYS		
				DOMINANT	RECESSIVE	DIFFERENCE
ddYYJJTTUUW^{t}W^t		Tiny Tim (P-1)	90	9.88	—	—
DDyyjjttuuw^{t}w^t		Pennorange (P-2)	89	4.76	—	—
DdYyJjTtUuW^{t}w^t		F_1	90	6.72	—	—
Standard: dwarf	1	BC to P-1	267	8.63	8.50	0.13
D : d		F_2	718	8.17	8.33	−0.16
Yellow: colorless	3	BC to P-2	264	6.84	7.05	−0.21
Y : y		F_2	718	8.00	8.73	−0.73*
Jointed: jointless	5	BC to P-2	264	7.57	6.56	1.01*
J : j		F_2	718	8.36	7.67	0.69*
Normal: wilty leaf	5	BC to P-2	264	7.39	6.84	0.55
W^t : w^t		F_2	718	8.43	7.67	0.76*
Red flesh: orange	7	BC to P-2	264	7.42	6.91	0.51
T : t		F_2	718	8.29	7.94	0.35
Green base: uniform	7	BC to P-2	264	7.93	7.10	0.83
U : u		F_2	718	8.44	7.81	0.63

* Sig. diff. (5%).

chromosome seven. On chromosome three, shorter duration is associated with yellow skin color from Tiny Tim but on the other two chromosomes with Pennorange markers. The gene for shorter period associated with yellow skin color gave a highly significant decrease in number of days for the period if the other three genes were for shorter period but no decrease if the other genes were for longer period. An interaction approaching significance was noted in similar comparisons of *Jj* in the backcross to Pennorange. Other comparisons were not significant. Effects of the genes for length of this period do not seem strictly additive.

The partitioning method suggests three complementary gene pairs for

shorter period from Pennorange and one from Tiny Tim having a greater effect than each of the Pennorange genes. Arithmetic inheritance and slight dominance of shorter period were suggested. Associations of shorter period with recessive marker genes on chromosomes five and seven and with the dominant on chromosome three were found. A fourth gene suggested by partitioning was not found in the association tests, and effect of the genes did not seem entirely additive.

Number of Days from Fruit Setting to Fruit Ripening

Results on this stage of growth are given in table 10. Tiny Tim was twelve days earlier than was Pennorange. High genetic variances for the backcrosses and F_2 in relation to the parents and F_1 suggest that the parents were homozygous. Partial dominance of shorter duration is also suggested.

Three classes of approximately equal intervals give best differentiation of parental and F_1 populations, as shown in the last three columns of table 10.

TABLE 10

Individual plant means, variances, and condensed frequency distributions for number of days from first fruit set to first fully ripe fruit.

GENERATION	NO. OF PLANTS	MEAN NUMBER DAYS	VARIANCES			FREQ. DISTRIBUTION		
			TOTAL	ENVIRON.	GENETIC	24–39	40–49	50–60
Tiny Tim	90	35.70±0.28	6.87	5.79	1.08	84.5	15.5	0.0
BC to P-1	269	37.30±0.23	13.69	6.17	7.52	66.5	32.7	0.8
F_1	90	37.92±0.24	5.31	6.31	−1.00	63.4	36.6	0.0
F_2	717	41.01±0.18	18.04	7.03	11.01	27.8	68.8	3.4
BC to P-2	267	43.13±0.27	19.74	7.53	12.21	10.3	81.4	8.3
Pennorange	89	47.35±0.32	8.44	8.52	−0.08	0.0	74.3	25.7

The F_2 penetrance in the 50–60 day class was 13.23 percent and the backcross to Pennorange 32.39 percent that of Pennorange. An hypothesis of two major factors dominant in Tiny Tim and a relatively minor one from Pennorange gave good agreement to observed penetrances in the segregating generations. One of the dominants from Tiny Tim may have a greater effect than the other since there was an excess in the 24–39 day class when they were assumed to have equal effect.

Table 11 shows significant decreases associated with standard plant type of Pennorange on chromosome one and with dark green base from Tiny Tim on chromosome seven. A third gene suggested by partitioning was not located. A significant interaction was noted in comparisons of dominant and recessive classes of the flesh-color gene when the plant type and uniform color loci were dominant or recessive. Significant interactions were not shown in comparisons of the *Dd* and *Uu* loci.

Three major gene pairs for duration of this stage were suggested by partitioning and two by association tests. Shorter duration showed dominance

TABLE 11

Mean number of days from first fruit set to first fully ripe fruit of plants in populations segregating for genes used to mark four chromosome pairs.

GENOTYPE OR PHENOTYPE	CHROMOSOME PAIR	GENERATION	NO. OF PLANTS	NUMBER OF DAYS		
				DOMINANT	RECESSIVE	DIFFERENCE
ddYYJJTTUUW^{t}W^t		Tiny Tim (P-1)	90	36.27	—	—
DDyyjjttuuw^{t}w^t		Pennorange (P-2)	89	47.89	—	—
DdYyJjTtUuW^{t}w^t		F_1	90	38.38	—	—
Standard: dwarf	1	BC to P-1	269	37.14	38.60	−1.46**
D : d		F_2	717	41.31	42.32	−1.01**
Yellow: colorless	3	BC to P-2	267	43.36	44.02	−0.66
Y : y		F_2	717	41.55	41.38	0.17
Jointed: jointless	5	BC to P-2	267	44.11	43.36	0.75
J : j		F_2	717	41.45	41.78	−0.33
Normal: wilty leaf	5	BC to P-2	267	44.01	43.31	0.70
W^t : w^t		F_2	717	41.61	41.22	0.39
Red flesh: orange	7	BC to P-2	267	43.27	44.01	−0.74
T : t		F_2	717	41.38	41.89	−0.51
Green base: uniform	7	BC to P-2	267	42.89	44.03	−1.14*
U : u		F_2	717	40.99	43.11	−2.12**

* Sig. diff. (5%).
** Sig. diff. (1%).

over longer duration of the period. The effects of the genes did not appear strictly additive in association studies.

Number of Days from Seeding to Fruit Ripening

The complex nature of the data on the entire earliness period limits conclusive statements as to its inheritance. Too many genes affect the rate of development to make use of the partitioning method in any detail and too few qualitative genes were used in the cross to obtain extensive results from association or linkage tests. By considering results on the three component periods it seems evident that all chromosomes marked are effective on the rate of development. It would seem quite possible that additional genes occur on other chromosome pairs.

DISCUSSION

Fruit weight and earliness, and the components of each, were studied in a cross of the tomato varieties Tiny Tim and Stemless Pennorange. The field

planting included both parents, F_1, F_2, and backcrosses of the F_1 to each parent in a randomized complete block design. Two types of analyses were applied to the same data in an effort to estimate as nearly as possible the number of major gene pairs differentiating the parents and the nature of dominance and interactions. The first is a type of genotypic analysis termed the partitioning method and the second, association of a quantitative character or component with qualitative characters used as chromosomal markers. Segregation of certain qualitative characters thought to have simple inheritance was noted and six characters suggesting single factor inheritance were used to mark chromosome one, three, five, and seven in association studies.

Inheritance of both fruit weight and earliness appeared complex and separation of each into components was desirable. The fruit weight components, locule number and weight per locule, each were estimated to have segregated for three major gene pairs showing multiplicative action. Different gene pairs appeared to affect the two components but genes for each component were suggested on chromosomes three and seven. Fewer locules and smaller fruit size showed partial dominance but dominance was not apparent for the weight per locule component. A trend toward interallelic interactions was evident for each component.

Earliness was divided into three stages thought to be biologically different. Partitioning suggested five or more gene pairs affecting duration of the stage from seeding to flowering, four pairs for the period from flowering to fruit setting, and three pairs for the stage from fruit setting to fruit ripening. Association tests suggested four or more gene pairs for the first stage, three or more for the second, and two for the third period. Eight chromosome pairs were not marked by qualitative characters and associations were possible on chromosomes not marked or too distant from the marker gene to show association. At least five gene pairs and as many as twelve were thought to differentiate the parents for earliness. Failure of environmental variances to show the expected straight line relationship with means did not appear to be due to heterozygosity of the parents. Effects of the genes approached additive progressions but deviations were noted.

Conclusions reached by the two methods of analysis do not disagree in general. The partitioning method appeared to have value in estimating the number of major gene pairs segregating in a cross and the general nature of dominance and interactions for characters or components affected by four or fewer gene pairs.

Simultaneous selection for larger locule number and greater weight per locule would be expected to give quickest results in recovering fruit weight reduced by crosses involving a wild relative or other small-fruited types. Partial dominance of small fruit weight and geometric action of fruit weight genes tend to produce fruit weight approaching that of the smaller parent. Selection for both fruit weight components would assume importance therefore in advanced generation or backcross programs involving a small-fruited parent.

Partial dominance of earliness and the approximately additive nature of

earliness genes suggest that breeding for earliness may be less complex than for large fruit weight. A larger number of earliness genes may segregate, however, requiring large segregating populations. Selection for earliness greater than the early parent, Tiny Tim, seemed possible by selection for shorter period from flowering to fruit setting.

SUMMARY

Inheritance of earliness and fruit size was studied in a cross between two dissimilar tomato varieties. For fruit weight the method of partitioning genotypes suggested three segregating genes for each of the components, number of locules and weight per locule. By association with certain simply inherited characters, genes for each component were suggested on chromosome pairs three and seven, for number of locules also on chromosomesone and for weight per locule on five in addition to three and seven.

The interval from seeding to fruit ripening was divided into three periods: seeding to flowering, flowering to fruit set, and fruit set to fruit ripe. By partitioning analyses, estimates of five or more differentiating gene pairs for the first stage, four for the second period, and three for the third, appeared valid. Of four chromosomes compared for associations, each had genes for the first period, two and possibly three for the second, and two for the third.

Multiplicative inheritance of each fruit weight component was suggested but effects of earliness genes were additive in nature. Partial dominance of smaller fruit size and earliness was shown.

LITERATURE CITED

BUTLER, L., 1936 Inherited characters in the tomato II. Jointless Pedicel. J. Hered. **27**: 25–26.

CURRENCE, T. M., 1938 The relation of the first chromosome pair to date of fruit ripening in the tomato (*Lycopersicum esculentum*). Genetics **23**: 1–11.

LINDSTROM, E. W., 1924 A genetic linkage between size and color factors in the tomato. J. Mich. Acad. Sci. XL: 1574.

MACARTHUR, J. W., 1934a Fruit size effects of qualitative genes in the tomato. Amer. Nat. **68**: 73–74.

1934b Linkage groups in the tomato. J. Genet. **29**: 123–133.

1941 Size inheritance in tomato fruits. J. Hered. **32**: 291–295.

POWERS, LEROY, 1939 Studies on the nature of the interactions of the genes differentiating quantitative characters in a cross between *Lycopersicon esculentum* and *L. pimpinellifolium*. Genetics **39**: 139–170.

POWERS, LEROY, and C. B. LYON, 1941 Inheritance studies on duration of developmental stages in crosses within the genus Lycopersicon. J. agric. Res. **63**: 129–148.

POWERS, LEROY, L. F. LOCKE and J. C. GARRETT, 1949 Partitioning method of genetic analysis applied to quantitative characters of tomato crosses. U.S.D.A. Technical Bull. 998. 57 pp.

SINGH, DHARAMPAL, 1949 Inheritance of certain economic characters in squash, *Cucurbita maxima* Duch. Minn. agric. Exp. Sta. Tech. Bull. 186.

YEAGER, A. F., 1937 Studies on the inheritance and development of fruit size and shape in the tomato. J. agric. Res. **55**: 141–152.

EFFECT OF CONCENTRATION OF PANTOTHENATE ON SELECTION OF A MUTANT FOR PANTOTHENATE SYNTHESIS IN SACCHAROMYCES CEREVISIAE[1,2]

CAROLINE RAUT

Henry Shaw School of Botany, Washington University, St. Louis, Missouri[3]

Received November 28, 1949

CULTURE 2154, which was used in this study, is a haploid clone requiring pantothenate for growth. In the pedigree of which this culture is a member, whenever a pantothenate-independent strain is crossed with a dependent strain, the segregation obtained is always two dependent and two independent spores in each four-spored ascus. Hence, the requirement for pantothenate is determined by the segregation of a single gene (LINDEGREN and LINDEGREN 1947). Clone 2154 is further characterized by being a nonfermenter of both maltose and melibiose, and of mating type "a."

However, if this culture is held for a month or longer in medium containing no pantothenate, it acquires the ability to grow without added pantothenate. The time required for adaptation to occur is shortened by the addition of very small amounts of pantothenate, but is prevented entirely by the addition of larger amounts (fig. 1). This phenomenon is like that studied previously (LINDEGREN and RAUT 1947 a; b) with a strain which did not segregate regularly.

The effect of concentration of pantothenate on adaptation was tested using the following procedure: Kimble test tubes (150×18 mm) were prepared in duplicate containing 12 ml of BURKHOLDER's medium (1943) with concentrations of calcium pantothenate of 200γ, 100γ, 50γ, 20γ, 5γ, 2γ, 1γ, and 0γ per liter. The tubes were inoculated with around 1000 cells of culture 2154 and placed in an incubator held at 30°C. Turbidity readings were taken at intervals throughout the period of growth with a Klett photoelectric colorimeter adapted to accommodate the large sized tubes.

DEMONSTRATION OF A GENE FOR PANTOTHENATE SYNTHESIS AT A DIFFERENT LOCUS IN THE ADAPTED CULTURE

A culture of 2154 adapted after seven weeks in BURKHOLDER'S medium with no added pantothenate. On a second transfer to the same medium, it produced full growth in five days. After several transfers on the deficient medium, crosses were made between this culture (designated 2154+, adapted)

[1] Submitted in partial fulfillment of the requirements for the degree DOCTOR OF PHILOSOPHY in the HENRY SHAW SCHOOL OF BOTANY, WASHINGTON UNIVERSITY, St. Louis, Missouri. This work was carried out at WASHINGTON UNIVERSITY and at SOUTHERN ILLINOIS UNIVERSITY.

[2] This work was supported by grants from ANHEUSER-BUSCH, INC., AMERICAN CANCER SOCIETY and the U. S. PUBLIC HEALTH SERVICE.

[3] Present address: Microbiology Department, Southern Illinois University, Carbondale, Illinois.

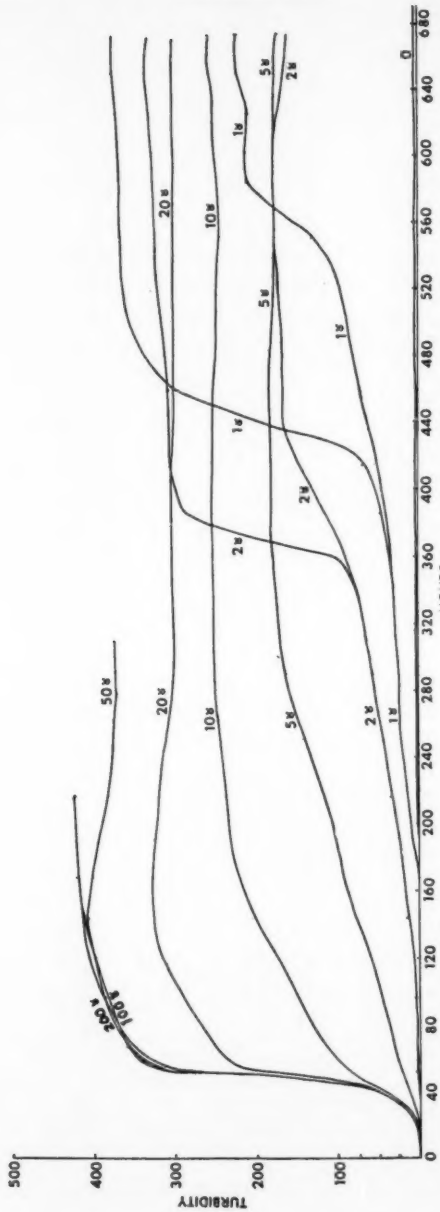


Figure 1.—Growth of pantothenate-dependent culure 2154 in the presence of various concentrations of pantothenate.

and dependent and independent clones from two different asci derived from a cross of an independent strain by a dependent unadapted strain. The four spore clones from each of the two asci were numbered 2113 (−), 2114 (+), 2115 (+), 2116 (−), and 2151 (−), 2152 (+), 2153 (+), 2154 (−), where + indicates ability to grow without added pantothenate and −, the inability.

Haploid cultures grown from isolated ascospores from these crosses were tested for ability to grow in BURKHOLDER'S medium containing no added pantothenate, as described in the previous section. A control test was always made by inoculating a tube of medium containing 200γ of pantothenate per liter. This made it possible to detect cases in which failure to grow was not due only to inability to grow without pantothenate, but to some additional deficiency. The culture marked with a single asterisk (table 1) failed to grow in the control tube containing pantothenate presumably due to its inability to synthesize some essential substance not present in Burkholder's medium.

The results obtained are given in tables 1 and 2.

TABLE 1

Growth in pantothenate-free medium of clones from asci of various hybrids.

PARENTS	2154 ad +[5]†	2154 −(>30)	2153 +[3]	2115 +[4]
2113 −(>40)	+[5] +[5] − −	− − − −		
	+[4] +[6] − −	− − − −		
	+[5] +[7] − −	− − − −		
	+[5] +[8] − −			
	+[4] +[5] −			
	+[7] −* −			
2114 +[4]	+[3] +[4] +[6] −	+[3] +[4] − −		+[2] +[3] +[4] +[4]
	+[5] +[5] +[11] −	+[3] +[3] − −		+[3] +[3] +[3] +[4]
	+[3] +[3] +[8] −			+[3] +[3] +[3]
2151 −(>30)	+[7] +[9] − −	− − − −		
	+[8] +[11] − −	− − − −		
	+[6] +[11] − −	− − − −		
	+[5] − −	− − − −		
	+[9] − −			
2152 +[3]	+[4] − +[7] +[4]	+[3] +[4] − −	+[5] +[5] +[6] +[10]	
	+[3] +[6] +[6] −	+[3] +[4] − −	+[4] +[4] +[4]	
	+[4] +[4] +[8] −	+[3] +[4] −	+[5] +[5]	
	+[4] +[4] +[6] −			
	+[3] +[4] − +[5]‡			
	+[3] +[5] +[6] −			
	+[4] +[4] − −			

† Superscript numerals indicate time in days to half-maximum growth. Numbers in parentheses indicate number of days before adaptation of pantothenate dependent cultures took place.

* Control tube containing pantothenate did not grow; apparently loss mutation involving some unspecified substance.

‡ 4175, 4176, 4177, 4178.

In the mating of culture 2154 (+ adapted) by the two non-synthesizing cultures, two spores, from each four-spored ascus, gave rise to pantothenate-independent clones which approached full growth in from four to eleven days in minus pantothenate medium, and the other two spores produced dependent clones. On the other hand, matings of unadapted 2154 (−) by the same two non-synthesizing cultures, 2113 (−) and 2151 (−), produced asci which contained only dependent spores. This clearly demonstrates that the adapted culture contains a single gene for pantothenate independence which is not present in the original non-synthesizing culture 2154.

In order to determine whether the mutation involved back mutation of the recessive allele to the dominant allele of the gene for pantothenate synthesis in this pedigree, or was a mutation at a different locus, crosses were made between 2154 (+, adapted) and both 2114 (+) and 2152 (+). If the mutation be at the same locus, only independent progeny will be obtained. If two different loci are involved, three kinds of asci may be obtained. If we designate one pair of alleles for independence, as *A* and *a*, and the other as *B* and *b*, the genotypes of the four spore clones which may be obtained in the three types of asci are (1) *AB*, *AB*, *ab*, *ab*, (2) *Ab*, *Ab*, *aB*, *aB*, and (3) *AB*, *Ab*, *aB*, *ab*. If the presence of either *A* or *B*, or both, will enable the organism to grow without added pantothenate, the first type of ascus listed will contain two independent and two dependent spores, the next all independent and the last, three independent to one dependent. If the genes are freely assorting, these asci will be obtained in a ratio of 1:1:4 (LINDEGREN 1949a, b).

A hybrid of synthesizer 2114 (+) by 2154 (+, adapted) produced three complete asci each containing three independent ascospores. If we designate the genotype of synthesizers in the parent stock as *Ab* and 2154 (+, adapted) as *aB*, the "double" synthesizer can be indicated by *AB* and the non-synthesizer by *ab*. These asci are then of the type designated as *AB*, *Ab*, *aB*, *ab*. A mating of 2114 (+) by 2154 (−, unadapted) confirmed the previously reported fact (LINDEGREN and LINDEGREN 1947) that the original synthesizer (*Ab*) stock contains a single synthesizing gene. The mating of 2114 (+) by 2115 (+) shows that the two carry the same allele, the cross of *Ab* by *Ab* producing four *Ab* synthesizers in each ascus. A mating similar to the first, of 2154 (+, adapted) by its synthesizing sister 2152 (+), produced six asci containing three synthesizers and one ascus containing two synthesizers. The asci are *AB*, *Ab*, *aB*, *ab*, and *AB*, *AB*, *ab*, *ab*, respectively. The third possible type *Ab*, *Ab*, *aB*, *aB*, did not appear. A mating of 2152 (+) by the unadapted 2154 (−) confirmed the fact that 2152 contained only a single gene affecting synthesis (*A*), and a mating of 2152 (+) by 2153 (+) verified that 2152 and 2153 each carry the same gene controlling the synthesis of pantothenate, since no spores incapable of synthesizing pantothenate were obtained.

This analysis makes it possible to assign the genotype *aB* to 2154 (+, adapted); 2153, 2114 and 2152 are *Ab*; 2154 unadapted, 2151, and 2113 are *ab*. Adaptation to pantothenate independence in 2154, thus, must have resulted from a mutation of *b* to *B*.

One of the asci obtained from the mating of 2152 and 2154 (+, adapted) was

subjected to further analysis shown in table 2. Cultures 4175, 4176, 4177, and 4178, from a single ascus, consisted of three independent clones and one dependent clone. 4176 (+) mated to 2151 (−) produced one ascus containing four independent spores, two asci containing three, and one complete ascus containing two. This is clearly a mating of a clone containing two independent genes by a clone with both dependent alleles, *AB* by *ab*. Therefore, 4176 has

TABLE 2

Growth in pantothenate-free medium of clones from asci obtained in backcrosses of 4175 (+), 4176 (+), and 4178 (+), to recessives.

PARENTS	4175 $+^3$	4176 $+^4$	4178 $+^5$
4064 −	$+^3\ +^4\ -\ -$		
2152 −(>30)		$+^3\ +^3\ +^5\ +^{11}$ $+^3\ +^5\ +^6\ -$ $+^4\ +^4\ +^6\ -$ $+^3\ +^3\ -\ -$ $+^3\ +^6\ -$ $+^5\ +^6\ -$	
4177 −			$+^{10}+^6\ -\ -$ $+^5\ +^5\ -\ -$ $+^4\ +^7\ -\ -$

the genotype *AB*. Mating of the two other independent clones, 4175 and 4178, by dependents, produced asci indicating that 4175 and 4178 each contained a single independent allele. The clones 4175, 4176, 4177, 4178, from the single ascus analyzed, can be designated as *Ab*, *AB*, *ab*, and *aB*, respectively. Cultures 4175 and 4178 both carry a single gene for independence and are either *Ab* or *aB*.

The two genes for pantothenate synthesis can be distinguished roughly by the growth rates of the clones bearing these genes. The original synthesizers (*Ab*) usually begin the observable rapid phase of growth in pantothenate-free medium before the adapted 2154 genotype (*aB*). Among the asci in which 3:1 segregations occurred, two of the clones usually grew more rapidly than the third (table 1). Hence, apparently, the clone of the genotype *AB* and that of the genotype *Ab* generally grow at the same rate while the genotype *aB* grows more slowly. On this basis, 4175 has been designated *Ab* and 4178 *aB*. Figure 2 shows growth curves for yeast bearing the *B* gene (2154 adapted) on a series of concentrations of pantothenate. The adapted strain (*aB*) grows more slowly in high concentrations of pantothenate (e.g. 200γ per liter) than does the original unadapted 2154 (*ab*). The *ab* clones showed no evidence of growth for over a month, but all were subject to the adaptation phenomenon described in detail for culture 2154.

To determine if the *B* gene controls the synthesis of pantothenate, culture

2152 (*Ab*), which synthesizes pantothenate, and adapted 2154 (*aB*), were assayed for pantothenate after growth in minimal medium. Both cultures were digested in 10 ml distilled water at pH 5.5 in the autoclave at 20 pounds pressure for 15 minutes. The extracts thus prepared were assayed with culture 2154, as follows. Increasing amounts of the extract were added to test tubes containing 10 ml of BURKHOLDER'S synthetic medium without added pantothenate. Each tube was inoculated with around 1000 cells of culture 2154, the

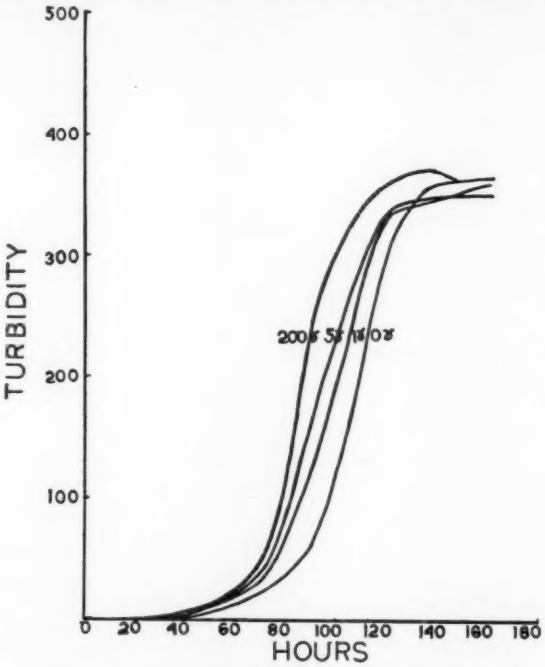


FIGURE 2.—Growth of adapted culture 2154 in a series of concentrations of pantothenate.

tubes were incubated at 30°C and the amount of growth determined photometrically as described previously.

Unadapted culture 2154 was used as the assay organism, since it differed from the adapted culture only in the ability to grow in the absence of pantothenate, and thus the autolysate should not contain any growth factors not in the assay organism except for the factor responsible for ability to grow in the absence of pantothenate.

Increasing concentrations of extracts from both cultures produced an increase in both the rate and the final amount of growth, thus indicating that the adapted (*aB*) strain, as well as the original (*Ab*) strain, is able to synthesize pantothenate.

THE RANDOM BASIS OF MUTATION TO SYNTHESIS

To study the nature of the adaptation in pantothenate-free medium, duplicate tubes of culture 2154 were grown as before in synthetic medium with no added pantothenate and followed closely to determine the number of cells present when the adaptation occurred and what proportion of the cells were adapted. Samples were taken and the cells counted with a haemocytometer at frequent intervals during the growth period to determine if a change in growth rate occurred; at the same time samples were plated on plus and minus pantothenate agar plates to detect the presence of mutants.

One of the duplicate tubes did not begin to show appreciable visible growth until about 1000 hours (46 days) and then exhibited a generation time of about 40 hours. The other tube began to show appreciable growth at around 1900 hours (80 days) and had a generation time of around 75 hours during the rapid phase of growth. The generation time of a fully adapted culture after several transfers on minus pantothenate medium is about three to four hours during the rapid growth phase. Evaporation of water from the tubes was not corrected for, but this error was not appreciable until late in the growth of the culture.

Figure 3 shows the number of cells in one of the two cultures grown about three months at 30°C, plotted on semilogarithmic graph paper. While there is fairly close fit of all the points plotted during the logarithmic growth phase of the culture, the inoculum does not fall on the line. This indicates either a long lag before growth begins or a change in the rate of growth from that of the original inoculum, after which the rate remains constant with a generation time of about 75 hours.

Considerable growth had occurred before any mutants were detected in the samples plated. Rapidly growing mutant colonies, which appeared in about 12 days on minus-pantothenate agar plates, were isolated from the culture tube at the points indicated (fig. 3). From the 40,000 to 70,000 dependent colonies which were tested 97 and 102 days after inoculation (as estimated from samples plated on complete agar plates) one large independent colony grew on each of the pantothenate-free agar plates streaked on the two days. It appears that the culture grows slowly in the pantothenate-free medium and thus reaches a size such that mutation to pantothenate synthesis has a very high probability of occurring. The mutant cells may or may not replace the parent cells in the population depending on the time of occurrence of the mutation. The duplicate of this culture and a third culture studied similarly, also produced considerable growth before mutants were detected in the samples plated.

Prior to the appearance of the two rapidly growing colonies, some of the cells plated on pantothenate-free agar grew slowly and formed visible colonies (around 0.25 mm) in about two weeks. Thirty of these colonies were tested by transfer into pantothenate-free liquid medium, where, except for five colonies which did not grow, they showed considerable growth (a turbidity reading of between 60 and 270) in about nine days. Because of their slow growth, a good many such colonies were probably overlooked. Apparently pantothenate-

independent "mutants" with intermediate growth rates occurred in addition to the rapidly growing mutants detected at the end of growth.

A series of experiments was carried out to determine whether or not the mutations to independence occur at random in the absence of pantothenate.

A few hundred cells from separate colonies of culture 2154, isolated from the complete medium plates which were used for counts in figure 2, were inoculated into small tubes containing 5 ml of pantothenate-free medium and were allowed to grow for over a month. At this time appreciable growth had occurred and the entire cultures were plated out on minus-pantothenate agar plates to determine the number of mutants in each. Table 3 gives the results of

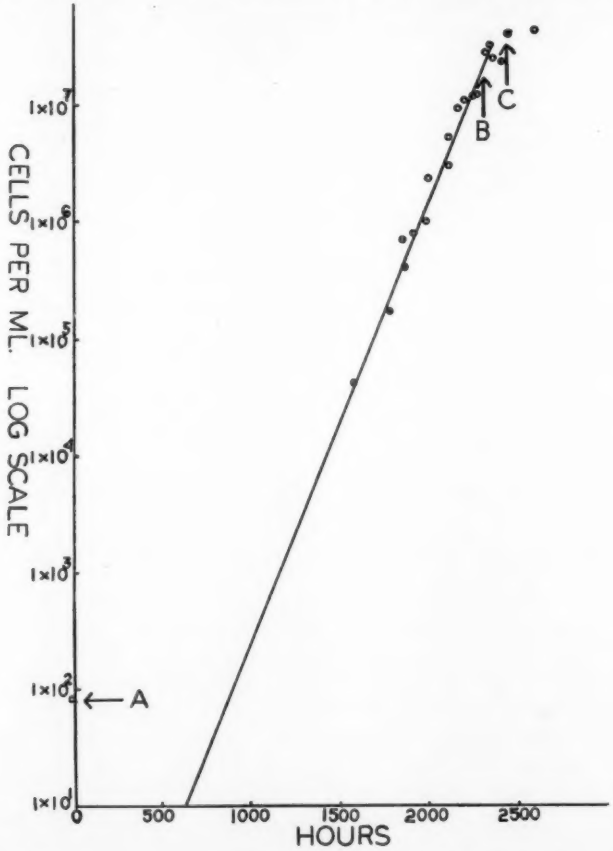


FIGURE 3.—Growth of pantothenate dependent culture 2154 in pantothenate-free medium. Arrow A indicates the inoculum and arrows B and C the points where rapidly growing mutants were detected. These data are interpreted as indicating that the yeast cells grew slowly in the pantothenate-free medium before mutations to the final "adapted" form occurred.

a typical experiment. This experiment was repeated several times with similar results each time.

TABLE 3

Occurrence of mutants in pantothenate-free medium.

TUBE	TOTAL CELLS	DAYS TO APPEARANCE OF COLONIES						
		3	7	11	16	18	25	31
		COLONIES						
1	1.4×10^6	1	2	2,1s	3	3,30s	3,10s	3,15s,nu
2	17.3×10^6	0	3<50vs	4<50vs	4<50vs	4<50s	4<50s	4,77s,nu
3	3.2×10^6	0	0	50vs	50vs	50s	5<100s	5<100s
4	5.6×10^6	0	0	0	1s	1s,nu	1s	1s
5	9.2×10^6	0	0	0	1<1,000vs	4<1,000vs	7<1,000vs	4,115s 4800vs
6	16.7×10^6	0	0	0	nu	nu	nu	nu
7	17.3×10^6	0	0	0	nu	7s<1,000vs	9<1,000vs	9<320vs
8	25.8×10^6	0	0	0	0	0	0	0
9	4.3×10^6	0	0	0	4	4	4, nu	7, nu
10	1.8×10^6	0	0	0	1,nu	1<100vs	1<100vs	7<100vs
Av.	10.3×10^6							

Numbers alone designate large colonies, ranging from approximately 0.4 to 0.6 mm in diameter. The letters s and vs designate small and very small colonies. 3,3s means there are 3 large and 3 small colonies on the plate; "nu" means "not uniform." The film of yeast cells on some of the plates appeared uneven although there were no colonies visible macroscopically. Microscopic examination revealed that a considerable number, around a fifth, of the colonies had reached a size of several hundred cells; these plates were classified "nu."

Since, in the absence of pantothenate, selection favors the synthesizing mutants, the variation in number of cells in a mutant clone due to difference in time of mutation is magnified. For this reason, a comparison of the variance among plates made from different tubes with the variance among plates of samples taken from the same tube would be biased. However, from the great variation in the number of mutant colonies, it appears clear that the mutations occur by chance (LURIA and DELBRÜCK 1943). From the variation in the sizes of the colonies it seems that growth rates of the mutants are not all the same.

Since a number of days elapsed before colonies appeared on the plates, there is a possibility that mutations may have occurred on the plates. For this reason, complete data are given to show the variation in time of appearance of the colonies on the plates. For plates with large numbers of mutant colonies, exact counts were made at the end of the experiment; the intermediate numbers given in the table are estimates. It is important to note that for the most part, the colonies appeared simultaneously on any one plate.

Experiments were performed to determine the validity of the plating method by mixing adapted and unadapted cells. Around 1000 cells of culture 2154 were inoculated into each of four tubes of 20 ml of BURKHOLDER's medium contain-

ing 200γ of pantothenate per liter. After growing to near completion, the yeast was washed five times with sterile distilled water and yeast from each tube divided into five equal lots in separate sterile test tubes. A series of dilutions of a suspension of mutant 2154 cells was added to the tubes as follows: (1) no mutant cells, (2) 1×, (3) 10×, (4) 100×, (5) 1000×. The mixtures of cells were then plated on pantothenate-free agar plates. The results are given in table 4.

TABLE 4

Number of colonies produced by pantothenate independent mutant cells mixed with non-mutant dependent cells and plated on minus pantothenate agar plates.

TUBE	A	B	C	CONTROL
NO. PARENT CELLS/PLATE	4.6×10^7	5.8×10^7	1.6×10^8	0
DILUTION OF MUTANT CELLS ADDED	NUMBER OF COLONIES IN 7 DAYS			
1000×	156	148	16	175
100×	> 1,000	> 1,000	> 100	> 1,000
10×	> 10,000	> 10,000	>1,000	> 10,000
1×	> 10,000 (>100,000 in 10 days)	>100,000	>1,000	>100,000
NO MUTANTS	0	1	0	—
	NUMBER OF COLONIES IN 16 DAYS			
NO MUTANTS	6	8	>1,000*	—
	TUBE D			
NO. PARENT CELLS/PLATE	1.2×10^8	NO. MUTANT CELLS ADDED:		0
Plate	(1)	(2)	(3)	(4)	(5)	(6)
Colonies in 7 days	0	0	0	0	0	0
Colonies in 16 days	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000

* More than 1000 colonies also developed in 16 days in the other four plates from tube C to which mutant cells had been added, indicating that these independent cells were present in the parent culture plated, and thus must have resulted from a mutation which occurred during the growth of this culture prior to plating. A comparable number of small colonies appeared between the large colonies in the 1000× dilution plates of cultures A and B at the end of this same time. Since no such colonies developed on the plates of these two cultures to which no mutant cells were added, these small colonies appeared to be non-mutant colonies growing on pantothenate produced by the large synthesizing colonies. A circle of such small colonies is frequently observed surrounding contaminant mold colonies, which apparently are able to synthesize pantothenate.

It is clear from a comparison of tubes *A* and *B* with the control containing no parent cells, that a preponderance of the mutant cells form colonies when added to populations of 4.6×10^7 and 5.8×10^7 non-mutant cells. However, the development of mutant colonies is suppressed in plates made from tube C. The culture C plates had a larger population of non-mutant cells, 1.6×10^8, and in addition contained a large number of independent cells which apparently developed from a mutation which occurred some time during the growth of the culture. Comparison of the plates to which no mutant cells were added indicates that while there is great variation between different tubes (A, B, C, and D) there is little variation between a series of plates made from a single tube (D).

The mutants which were added formed colonies within a shorter time than the mutant cells present in the cultures plated, which had arisen during the growth of these cultures. This is to be expected, however, since the mutant cells which were added came from a culture which had been selected by serial transfer on minus-pantothenate medium for rapid growth.

The possibility that mutations may occur on the plates has not been eliminated. In fact, the six and eight colonies which developed in cultures A and B, (with no added mutants) appeared one or two at a time over a period of nine days and thus may have been the result of mutations occurring on the plates.

In view of this, it is not possible to conclude that the number of colonies appearing on the plates in the previous experiment is the exact number of mutant cells in the predominantly non-mutant cultures plated. However, it appears that a valid distinction can be drawn between widely different numbers of mutant cells in a population of predominantly non-synthesizing cells. While the possibility of mutations occurring on the plates has not been eliminated, as may be observed from table 3, there were a number of instances in which 50 or 100, or even more colonies appeared simultaneously on one plate. This could be due to a large number of mutations occurring within a short time on the plate, but it seems much more likely that a large number of mutant cells were present in the inoculum plated.

It has been shown that growth takes place in synthetic medium to which no pantothenate has been added. It has also been shown that mutations to pantothenate synthesis occur by chance in the absence of pantothenate. These two facts could account for the adaptation of cultures held for a long time in medium containing no pantothenate or minute amounts of pantothenate. Since adaptation does not occur in intermediate concentrations of pantothenate, the question is raised as to whether mutations are suppressed by intermediate and high concentrations of pantothenate. (The experiment to verify the method indicates that mutations to synthesis do occur in cultures grown in complete medium.)

To determine this, two groups of test tubes containing 4 ml of complete medium (200γ of pantothenate per liter) were inoculated with around 500–1000 cells of 2154 and allowed to grow to completion. The yeast was then washed five times with sterile water and the entire cultures plated out on pantothen-

ate-free agar. The plates were incubated at 30°C and observed for appearance of colonies. The results obtained are given in table 5.

TABLE 5

Occurrence of mutants in the presence of pantothenate.‡

EXPERIMENT I*		EXPERIMENT II†			
TUBE	NO. COLONIES IN 23 DAYS	TUBE	NO. COLONIES IN 21 DAYS	TUBE	NO. COLONIES IN 21 DAYS
1	4,8vs	1	1,6s	12	4,5vs
2	3s	2	108s	13	0
3	2,4vs	3	6s	14	130vs
4	1,7s	4	9vs	15	1s
5	1,4vs	5	13s	16	0
6	1	6	4s	17	0
7	1s,9vs	7	24	18	3,34vs
8	1,4s	8	180s	19	340s
9	7	9	1s	20	3vs
		10	0	21	2,41s
		11	4,970s	22	11,717vs

* Average number of cells per plate—5.78×10^7.

† Average number of cells per plate—3.7×10^8.

‡ Designations the same as in table 3.

It appears from the variation in number of mutant cells, that mutation occurs by chance in both high and low concentrations of pantothenate, but because of the selection against the mutants in high concentrations, they are not ordinarily encountered.

Only summary data are given in the table, but, as in table 3, the number of colonies on the separate plates did not increase for the most part, after their original appearance on the plates.

COMPETITION BETWEEN MUTANT AND PARENT CELLS IN LIMITING CONCENTRATIONS OF PANTOTHENATE

To determine if competition between parent and mutant cells could account for the fact that adaptation does not occur in concentrations above 2γ of pantothenate per liter, a series of duplicate tubes were set up containing 200γ, 20γ, 10γ, 2γ, and 0γ of calcium pantothenate per liter and inoculated with a mixed inoculum of cultures 2154 and adapted 2154. The inoculum consisted of about 125 adapted cells and around 500 unadapted. The cultures were incubated at 30°C and growth measured by turbidity readings as before. Figure 4 shows the growth obtained as measured in colorimeter readings. The cultures were observed for another two weeks beyond the time shown on the graph, during which no appreciable change in the amount of growth occurred.

Rate and total amount of growth of the mixed cultures decreases as the con-

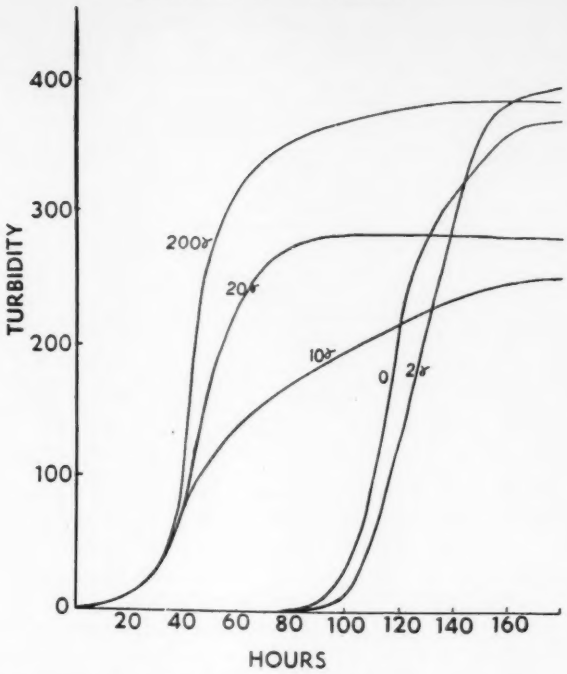


FIGURE 4.—Growth of mixed cultures 2154 and adapted 2154 in various concentrations of pantothenate. (Comparison of figure 4 and table 6 shows a divergence between the total number of cells and the turbidity readings in the intermediate concentrations. This is because in limiting concentrations of pantothenate the unadapted cells are small and thus the same number of cells produces less turbidity.)

centration of pantothenate decreases from 200γ to 10γ, but as it is dropped to 2γ and 0γ, there is a lag of four days after which rapid growth ensues. The total amount of growth is greater at 2γ and 0γ than at the intermediate amounts of 20γ and 10γ. When both types of cells are present, the non-synthesizing cells determine the growth in high concentrations of pantothenate while in very low concentrations the mutant cells make up the bulk of the population. It may be noted from table 6 that the total number of mutant cells in the mixed cultures at completion of growth increases as the concentration of pantothenate decreases. The ratio of the two types of cells in the final growth obtained was determined by plating samples on plus- and minus-pantothenate plates and the total number of cells estimated from haemocytometer counts.

If we refer to the growth rates of these two cultures grown separately, it is apparent from comparison of figure 1 showing growth rates for pantothenate-dependent strain 2154 alone and figure 2 for adapted 2154 alone, that the rate

of growth of the dependent strain varies extremely with concentration of pantothenate while the rate of the independent strain varies only slightly. The unadapted dependent strain begins to grow sooner and grows more rapidly than the adapted strain at high concentrations. In low concentrations of pantothenate the adapted strain grows more rapidly. In intermediate concentrations the growth rates of the two strains approach each other. Hence, in mixed

TABLE 6

Analysis of mixed cultures.

PA. CONC.	TOTAL CELLS	TOTAL MUTANTS	RATIO MUTANT:PARENT
200γ	2.1×10^8	1.9×10^4	1:11,000
20γ	1.6×10^8	6.2×10^4	1: 2,600
10γ	7.5×10^7	9.9×10^4	1: 760
2γ	1.6×10^8	1.6×10^8	76: 1
0γ	1.4×10^8	1.4×10^8	700: 0

cultures, while there is a possibility of competition between the cells of the two strains in all concentrations, it is effective only at intermediate concentrations, because at high concentrations the parent cells almost completely overgrow the mutant, while at low concentrations the mutant cells predominate. In other words, the more parent cells, the greater the inhibition of mutant cells. Either exhaustion of the medium or accumulation of by-products by the parent cells during their abnormal growth on limiting concentrations of pantothenate could inhibit growth of the mutant cells and thus account for the fact that maximum growth of mixed cultures is not obtained in intermediate concentrations. However, at the 2γ and 0γ levels, very little growth of non-synthesizing cells can take place, so the mutant cells attain approximately the maximum amount of growth. There appears to be slight inhibition of mutant cells even at 2γ.

This inhibition of growth of mixed cultures in intermediate amounts of the essential growth factor appears to be similar to that studied by RYAN and SCHNEIDER (1948) of the interaction between histidineless and histidine independent cells of *Escherichia coli*.

SUMMARY

Adaptation of a haploid yeast from pantothenate dependence to independence was found to be due to mutation of a single gene. The mutation is not a back mutation to the dominant allele present in the pedigree studied, but is at a different locus, as identified by the independent segregation of the wild-type and the mutant synthesizer.

Mutation to synthesis occurs by chance both in the presence and in the absence of pantothenate. However, in the presence of intermediate or high concentrations of pantothenate, the non-synthesizing cells overgrow the mutants and, therefore, adaptation occurs only in very low concentrations of

pantothenate. The dependent culture is able to grow very slowly in the pantothenate-free medium after a long lag and thus is able to reach a population size such that mutation is likely to occur.

ACKNOWLEDGMENTS

The author would like to express her appreciation of the encouragement and help given by DR. and MRS. CARL C. LINDEGREN during the course of this study, and also to the very helpful criticisms and discussions by DR. J. O. LAMPEN, DR. S. E. LURIA, and DR. FRANCIS J. RYAN.

LITERATURE CITED

BURKHOLDER, P. R., 1943 Vitamin deficiencies in yeasts. Amer. J. Bot. **30**: 206–211.

LINDEGREN, CARL C., 1949a The Yeast Cell, Its Genetics and Cytology; introd.+366 pp. St. Louis: Educational Publishers, Inc.

1949b Chromosome Maps of Saccharomyces. Eighth International Congress of Genetics. Hereditas, Lund. Suppl. Vol., 1949.

LINDEGREN, CARL C., and G. LINDEGREN, 1947 Mendelian inheritance of genes affecting vitamin-synthesizing ability in *Saccharomyces*. Ann. Mo. bot. Gdn. **34**: 93–99.

LINDEGREN, CARL C., and C. RAUT, 1947a The effect of the medium on apparent vitamin-synthesizing deficiencies of microorganisms. Ann. Mo. bot. Gdn. **34**: 75–84.

1947b A direct relationship between pantothenate concentration and the time required to induce the production of pantothenate-synthesizing "mutants" in yeasts. Ann. Mo. bot. Gdn. **34**: 85–94.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations in bacteria from virus sensitivity to virus resistance. Genetics **28**: 491–511.

RYAN, F. J., and L. K. SCHNEIDER, 1948 The consequences of mutation during the growth of biochemical mutants of *Escherichia coli*. J. Bact. **56**: 699–708.

Survey of Biological Progress

Volume I, 1949, 396 pages, illustrated $6.80

Edited by GEORGE S. AVERY, JR.
Brooklyn Botanic Garden, Brooklyn, New York

The annual Survey of Biological Progress has been founded for the purpose of informing workers in the various branches of biology of the state and advances of other branches, particularly as they bear on the progress of this science as a whole.

The series will cover all major phases of biology over a three-year period and will provide a medium for integrated presentation of facts and thoughts from the entire biological field. This will offset to a certain extent the isolating effect of rapidly increasing specialization.

CONTENTS

DONALD P. COSTELLO, Growth and Development—HARRIET B. CREIGHTON, Teaching Biology Today—MARTIN D. KAMEN, Tracer Methods in Biological Research—PHILIP R. WHITE, Growth Hormones and Tissue Growth in Plants—LINDSAY M. BLACK, Virus Tumors in Plants and Animals—ROBERT K. BURNS, Hormones and the Differentiation of Sex—G. E. HUTCHINSON AND EDWARD S. DEEVEY, JR., Ecological Studies on Pouplations—W. A. FRAZIER, Newer Methods in the Rapid Development of Disease-Resistant Vegetables—KARL E. MASON, Nutrition and Reproduction—KARL C. HAMNER, Influence of Environmental Factors on the Vitamin Content of Food Plants—BENTLEY GLASS, The Genes and Gene Action—Author Index—Subject Index.

IN PREPARATION

Advances in Genetics

Volume III, Approx. 280 pages, June 1950

Edited by M. DEMEREC
Carnegie Institution, Cold Spring Harbor, N.Y.

CONTENTS

N. H. HOROWITZ, Biochemical Genetics of Neurospora—E. B. LEWIS, The Phenomenon of Position Effect—A. R. G. OWEN, The Theory of Genetical Recombination—FREDERICK D. RICHEY, Corn Breeding—ESKO SUOMALAINEN, Parthenogenesis in Animals—BERTHE DELAPORTE, Observations on the Cytology of Bacteria—Author Index—Subject Index.

ACADEMIC PRESS INC., PUBLISHERS
NEW YORK 10, N.Y.

LA CELLULE

Recueil de Travaux originaux de Cytologie, Biologie et Histologie générale. Fondé par J. B. Carnoy en 1884. Publié par P. Debaisieux et P. Martens, Louvain (Belgique).

Trois fascicules constituent un tome, généralement annuel, de 400 à 450 pages in quarto, avec nombreuses planches hors-texte et des figures de texte. Prix de souscription: Frs belges 550, port inclus. Adresser les souscriptions à l'éditeur: Librairie Universitaire, rue de la Monnaie, Louvain. Pour toute autre communication s'adresser au Prof. P. Martens, rue Marie-Thérèse, 23 Louvain (Belgique).

JOURNAL OF GENETICS

Editor: J. B. S. HALDANE

FOUNDED in 1910 the journal publishes records of original research in Heredity Variation and allied subjects, and also from time to time articles summarizing the existing state of knowledge in the various branches of Genetics. All articles are illustrated both with text-figures, and plates, the latter in black and white, and colors.

Subscription price, per volume of 3 parts is 50s, net post free. Single parts 20s net, postage extra. Quotations for back numbers on application.

Orders to

CAMBRIDGE UNIVERSITY PRESS
LONDON: Bentley House, N.W.1.
NEW YORK: 51 Madison Ave.

ECOLOGICAL MONOGRAPHS

(Official Publication of the Ecological Society of America)

First issue published December, 1930

Managing Editors: A. S. PEARSE and H. J. OOSTING, *Duke University*

A quarterly journal devoted to the publication of original researches of ecological interest from the entire field of biological science. The journal will work in close cooperation with *Ecology* and will undertake the publication of papers of from 25 to 100 printed pages in length while *Ecology* will continue to specialize on papers of about 20 printed pages or less.

Published in January, April, July, and October

SUBSCRIPTION: $6.00

DUKE UNIVERSITY PRESS
COLLEGE STATION, BOX 6697
DURHAM, NORTH CAROLINA

Subscribe to Wistar Institute scientific periodicals for authentic research literature in the biological sciences and nutrition.

JOURNAL OF MORPHOLOGY
$20.00 domestic, $21.00 foreign

THE JOURNAL OF COMPARATIVE NEUROLOGY
$15.00 domestic, $16.00 foreign

THE AMERICAN JOURNAL OF ANATOMY
$15.00 domestic, $16.00 foreign

THE ANATOMICAL RECORD
$22.50 domestic, $24.00 foreign

THE JOURNAL OF EXPERIMENTAL ZOOLOGY
$22.50 domestic, $24.00 foreign

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY
$7.50 domestic, $8.00 foreign

JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY
$15.00 domestic, $16.00 foreign

THE JOURNAL OF NUTRITION
$22.50 domestic, $24.00 foreign

Send orders with remittance to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
36th Street and Woodland Avenue
Philadelphia 4, Pa.

BIOLOGICAL ABSTRACTS

Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in nine low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section A affords a very complete coverage of the Genetics literature—and it is priced at only $5.00 a year (Foreign $5.50).

Write for a sample copy.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia 4, Pa.

Stain Technology

A Journal for Microtechnic

The latest developments in staining and general microscopy are presented in two different ways:

Original articles, 30-40 each year.

Abstracts of articles appearing elsewhere, so worded that the technic may be followed without consulting the original paper.

Issued quarterly **$4.50 a year**
20-year index **$1.50**

Published 1946

5th Edition

Biological Stains

By H. J. Conn, Biological Stain Commission

Cloth, 5½ by 8¼ in.
346 pages

Price $4.25
(If cash accompanies order: $3.75)

For sale by
BIOTECH PUBLICATIONS
Lock Box 269, Geneva, N.Y.

ANNALS OF EUGENICS

A JOURNAL OF HUMAN GENETICS

Edited by L. S. PENROSE, assisted by JULIA BELL, R. A. FISHER, J. B. HALDANE, MARY N. KARN, R. R. RACE, J. A. FRASER ROBERTS

Annals of Eugenics, XV.2 March 1950

The familial distribution of diabetes mellitus: a study of the relatives of 1241 diabetic propositi. H. Harris

An analysis of the blood types and clinical condition of 2000 consecutive mothers and their infants. Kathleen E. Boorman

Hereditary factors in peptic ulcer. R. Doll & J. Buch

The blood groups of the Icelanders. Joyce A. Donegani, N. Dungal, Elizabeth W. Ikin and A. E. Mourant

The sex ratios in sibships with special reference to Geissler's data. H. O. Lancaster

On the family size in some hereditary disorders. S. M. de Delbue.

Rh type Ry in 3 generations. A. Johnstone

Weight and height of a population in 1943. W. F. F. Kemsley

Subscriptions, in advance, 50s. per volume.

Four parts, obtainable separately at 15s. each. Back volumes 55s. per volume.

Orders should be placed with

UNIVERSITY PRESS
CAMBRIDGE, ENGLAND

AMINO ACIDS AND PROTEINS

Cold Spring Harbor Symposia on Quantitative Biology

Volume XIV (1949), 232 quarto pages, 10 plates and numerous figures

Authoritative reviews of many aspects of recent investigations of amino acids and proteins, presented in 22 papers and edited discussions. Subjects considered are fractionation of proteins, protein hydrolysis, amino acid analysis, structure of proteins, metabolism and synthesis of amino acids and proteins. Table of contents sent on request.

Previous volumes still available: XI (1946) Heredity and Variation in Microorganisms; XII (1947) Nucleic Acids and Nucleoproteins; XIII (1948) Biological Applications of Tracer Elements.

Price per volume: $7.00 (postage extra; domestic 25¢, foreign 50¢).

Address: Biological Laboratory
Cold Spring Harbor, New York

NEW PUBLICATIONS RECEIVED

●

Giordano, Alfonso. Avviamento Alla Patologia Genetica dell'Uomo, Italy, Garganzi. 1949. XII + 165 pp. 2000 Lire.

Li, Ching Chun. An Introduction to Population Genetics. Peiping (China), National Peking University Press. 1948.

Survey of Biological Progress. Volume I. Edited by George S. Avery, Jr., New York, Academic Press, Inc. 1949. 396 pp. $6.80.

Simpson, George Gaylord, The Meaning of Evolution, a study of the history of life and of its significance for man. New Haven, Yale University Press. 1949. xvi, 365 p. 38 figures. O. $3.75. (The Terry Lectures).

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty-five printed pages (about twelve thousand words) except by special vote of the Editorial Board. In case unusually extensive tabular matter cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to cover the cost of reproducing colored and halftone plates and of printing expensive tables.

Authors should prepare their manuscripts so as to conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgements," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be typewritten separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

GENETICS furnishes 50 reprints, without covers, free. Covers and additional reprints may be secured at cost.

Manuscripts and editorial correspondence should be addressed to the Editor of GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

GENETICS Vol. 35 No. 3 MAY, 1950

CONTENTS OF PRECEDING ISSUES

SEPTEMBER, 1949

NEWCOMBE, HOWARD B., AND GRACE WORKMAN SCOTT, Factors responsible for the delayed appearance of radiation-induced mutants in *Escherichia coli*

HOULAHAN, MARY B., G. W. BEADLE AND HERMIONE GRANT CALHOUN, Linkage studies with biochemical mutants of *Neurospora crassa*.

CRARY, D. D., AND P. B. SAWIN, Morphogenetic studies in the rabbit. VI. Genetic factors influencing the ossification pattern of the limbs.

BROWN, WILLIAM L., Numbers and distribution of chromosome knobs in United States maize.

WHITE, M. J. D., A cytological survey of wild populations of Trimerotropis and Circotettix. (Orthoptera, Acrididae). I. The chromosomes of twelve species.

GREEN, M. M., A study of tryptophane in eye color mutants of Drosophila.

GARDNER, ELDON J., AND CHARLES M. WOOLF, Maternal effect involved in the inheritance of abnormal growths in the head region of *Drosophila melanogaster*.

IRWIN, M. R., On immunogenetic relationships between the antigenic characters specific to two species of Columbidae.

PERKINS, DAVID D., Biochemical mutants in the smut fungus *Ustilago maydis*.

STEPHENS, S. G., The cytogenetics of speciation in Gossypium. I. Selective elimination of the donor parent genotype in interspecific backcrosses.

NOVEMBER, 1949

ANDERSON, E. G., A. E. LONGLEY, C. H. LI, AND K. L. RETHERFORD, Hereditary effects produced in maize by radiations from the Bikini atomic bomb I. Studies on seedlings and pollen of the exposed generation.

FOX, ALLEN S., Immunogenetic studies of *Drosophila melanogaster* II. Interaction between the *vb* and *v* loci in production of antigens.

GEIRINGER, HILDA, Chromatid segregation of tetraploids and hexaploids.

HECHT, ADOLPH, Tetraploid ratios of self-sterility alleles: a correction.

HANSON, W. D., AND H. H. KRAMER, The genetic analysis of two chromosome interchanges in barley from F_2 data.

GERSH, EILEEN SUTTON, Influence of temperature on the expression of position effects in the scute-8 stock of *Drosophila melanogaster* and its relation to heterochromatization.

RUSSELL, ELIZABETH S., Analysis of pleiotropism of the W-locus in the mouse: Relationship between the effects of *W* and W^v substitution on hair pigmentation and on erythrocytes.

ROLLINS, W. C., S. W. MEAD, W. M. REGAN, AND P. W. GREGORY, The effect of inbreeding upon growth in Jersey cattle.

JANUARY, 1950

BEADLE, G. W., Rollins Adams Emerson.

CLAUSEN, R. E., and D. R. CAMERON, Inheritance in *Nicotiana tabacum*. XXIII. Duplicate factors for chlorophyll production.

WALTERS, MARTA SHERMAN, Spontaneous breakage and reunion of meiotic chromosomes in the hybrid *Bromus trinii* × *Bromus maritimus*.

WHITTINGHILL, MAURICE, Consequences of crossing over in oögonial cells.

GARDNER, ELDON J., and CHARLES M. WOOLF, The influence of high and low temperatures on the expression of tumorous head in *Drosophila melanogaster*.

OPATOWSKI, I., On the interpretation of the dose-frequency curve in radiogenetics.

MARKERT, CLEMENT L., The effects of genetic changes on tyrosinase activity in Glomerella.

STORMONT, CLYDE, Additional gene-controlled antigenic factors in the bovine erythrocyte.

Abstracts of Papers presented at the 1949 Meetings of the Genetics Society of America.

MARCH, 1950

KERR, WARWICK E., Genetic determination of castes in the genus Melipona.

MORGAN, DELBERT THOMAS, JR., A cytogenetic study of inversions in *Zea mays*.

BISHOP, CHARLES J., Differential X-ray sensitivity of Tradescantia chromosomes during the mitotic cycle.

HINTON, TAYLOR, A correlation of phenotypic changes and chromosomal rearrangements at the two ends of an inversion.

LINCOLN, RALPH E., and JOHN W. PORTER, Inheritance of *beta*-carotene in tomatoes.

DEMPSTER, EVERETT R., and I. MICHAEL LERNER, Heritability of threshold characters.

WAGNER, R. P., C. H. HADDOX, R. FUERST and W. S. STONE, The effect of irradiated medium, cyanide and peroxide, on the mutation rate in Neurospora.

NOVITSKI, E., The transfer of mutant genes from small inversions.

GECKLER, ROBERT P., Genetic changes induced by exposure to nitrogen mustard and their inheritance in *Paramecium aurelia*, Variety 4.

SINGLE NUMBERS $1.25 ANNUAL SUBSCRIPTION $6.00

obtainable from

BROOKLYN BOTANIC GARDEN, BROOKLYN 25, NEW YORK